PRACTICAL METHODS

OF

URINE-ANALYSIS

For Chemists and Druggists

WITH NOTES ON THE
COMPOSITION OF THE NORMAL AND ABNORMAL
RENAL SECRETIONS

SECOND AND ENLARGED EDITION

Published at the Offices of
THE CHEMIST AND DRUGGIST
42 CANNON STREET, LONDON, E.C.
BRANCH OFFICES; ADELAIDE, MELBOURNE, & SYDNEY, AUSTRALASIA
AND NEW YORK, U.S.A.
1902

PRINTED BY
SPOTTISWOODE AND CO. LTD., NEW-STREET SQUARE
LONDON

PREFACE

THE present edition of this book covers about the same ground as the first, but it has been in a great measure re-written, in order to emphasise essentials, and to render more clear the interdependence of many of the pathological conditions. The book is primarily a guide to chemists and druggists in a branch of work which is becoming more necessary to medical diagnosis, more precise and refined in its execution, and which therefore calls for the skill and experience of specialists. No class of men is better fitted to fill this office than are pharmacists. The book does not enter into speculative matter, nor does it trouble the beginner with obscure urinary ingredients of ill-defined or doubtful import, as it is believed that those who become exceptionally expert in urine-analysis, and wish to carry their experience farther, will find it advisable to consult the more pretentious works which are written for medical practitioners.

LONDON : *April*, 1902.

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OF

URINE-ANALYSIS

URINE IN HEALTH AND DISEASE

THE study of urine in disease is complicated to a great extent by the fact that urine in health is, between wide limits, variable in its composition. Moreover, a normal ingredient in solution may become an abnormal ingredient out of solution, or a normal ingredient, though variable in its quantity in health, may in certain circumstances indicate either by excess or diminution a serious pathological condition. In considering urine in health these facts must be borne in mind, and a very free interpretation must be given to any statement as to the approximate composition of normal urine. The following table may be taken as a fair representation of the chief ingredients and their relative and absolute proportions. It shows the usual average composition of a twenty-four hours' collection of normal urine secreted by a man :

Quantity . . .	40 to 50 fluid ounces	= 1,200 to 1,500 c.c.
Total solids . .	800 to 1,000 grains	= 54 to 70 grammes

The total solids consist of:

Urea . . .	350 to 600 grains	= 25 to 40	grammes
Uric acid . .	5 to 15 ,,	= 0·3 to 1	,,
Chlorine . . .	50 to 150 ,,	= 3·5 to 10	,,
Phosphoric acid .	30 to 60 ,,	= 2 to 4	,,
Sulphuric acid . .	20 to 60 ,,	= 1·3 to 4	,,

These, with their bases—sodium, potassium, ammonium,

calcium, and magnesium—also extractive matters, pigments, and traces of hippuric acid, mucus, and epithelial cells, make up the weight of total solids indicated above. In chemical analysis it is usual to consider the normal to fall within the following limits:

Urea	1·5 to 3 per cent.
Uric acid	0·3 to 0·7 part per 1,000
Phosphates as P_2O_5	2 to 3 ,, ,, ,,
Sulphates as SO_3	1·5 to 3 ,, ,, ,,
Chlorides as NaCl	5 to 10 ,, ,, ,,

Generally the urine of a woman is less in volume and the solids in solution less in quantity. A rough rule for calculating the daily urine excretion of a child is to double the age in ounces; thus a child aged 6 years passes about 12 oz. Diet and habit of life make a great difference both in the volume and the proportion of solids; thus a vegetable diet reduces the nitrogenous substances in a very marked degree.

Collection of Sample

This is often done in the most casual manner, even when a quantitative determination of one or more constituents is required. It is essential to any degree of accuracy that some trouble should be taken in this matter. The influence of diet is enough to make a vast difference in the nature of the urine in the same day, and certain pathological ingredients may be small in amount at one time and great at another. Generally it is best to submit to analysis a sample taken from the mixed excretion of twenty-four hours, and this is best obtained by emptying the bladder at some particular hour—say, 9 A.M.—and rejecting that portion, then collecting all that is passed in the next twenty-four hours up to and including 9 A.M. the following day. Mix the whole, measure it, and take a 6 or 8 oz. bottleful as a sample; mark it with the name of the patient, the date, and the quantity passed, thus:

A. B.
Date.....
Quantity.—67 oz.

Although this rule is essential in quantitative work, it is not always expedient in qualitative testing, as it is, for obvious reasons, irksome to a lady, while a man is often so busy about his work that he cannot be bothered with the collection, or may find it impossible to reserve one or two portions. A good plan in such circumstances is to take the urine of bedtime and the following morning, either separately or mixed. Sometimes the use of a catheter is necessary either to exclude discharges from other quarters than the bladder, or to ascertain the true seat of hæmorrhage and other abnormal matters that have been found in the urine.

Quantity

The quantity of urine excreted by a healthy man in twenty-four hours is, as already indicated, about 50 oz., by a woman rather less, and by a child about double its years in ounces. Habits of life influence the output to a great extent, particularly the quantity of fluid that is drunk, so that the volume may be reduced by half, or increased to double, and still come within physiological limits. Another factor which influences the amount is the skin : the more water lost by the skin in perspiration, the less passes out through the kidneys, therefore hot weather, exertion, or anything that produces sweating tends to the reduction of the quantity of urine. Conversely, cold, or anything that suppresses or reduces the skin's action, throws all the work of elimination upon the kidneys, and the volume is increased. These may be called physiological reasons for reduction or increase, but besides these there are pathological conditions which produce like results. Reduction of quantity is caused by fevers, from their action on the skin; diarrhœa and cholera, because of the watery stools; dropsical effusion drains water elsewhere; and obstruction in the urinary tract, by creating pressure backwards, checks the action of the kidneys. Diminution by these causes may be called mechanical. Then certain diseases of the liver impede the portal circulation, while some affections of the heart diminish arterial pressure, and these also may be said to act

mechanically. Another class of diseases, by destruction of the kidney-substance itself, such as acute nephritis and Bright's disease, prevent the proper action of the kidneys, and not only is water reduced in volume, but the other urinary constituents are diminished in amount.

Pathological increase is most notable in diabetes, accompanied with sugar in *diabetes mellitus* and without it in *diabetes insipidus*. A temporary increase is also observed in certain nervous disorders, particularly fits of hysteria. Occasionally there is an increase in some stages of structural disease of the kidney. The effect of diuretics must also be remembered. The patient generally first notices increase by frequent micturition and the necessity to rise at night to empty the bladder. Frequent micturition, however, must not be looked upon invariably as a sign of increased quantity of urine.

Translucency

Normal urine is transparent at the time of passing, but on standing a cloud of mucus and epithelial cells, detached from the surface over which the urine has passed, forms in it, and in about twelve hours sinks to the bottom. The description of the epithelial cells will be considered under the heading of Microscopical Examination. It is well here, however, to note that in women, particularly during an attack of leucorrhœa, the discharge from the vagina is often mingled with the urine and separates with the other sediment. Excepting the muco-epithelial cloud, all other constituents of normal urine should be in solution when it is passed. On standing, however, it may happen, without of necessity implying ill health, that some of the ingredients may separate. This is notably the case with urates after severe exertion or in cold weather—indeed, separation of urates is very common. They rapidly sink when the urine is at rest, and generally have a pink appearance, which has given rise to the term *brickdust deposit*. The pink colour is due to the crystals being pigmented by the urinary colouring-matter. Urates themselves when pure are not coloured. Sometimes red crystals like grains of Cayenne

pepper are thrown down. These are free uric acid. Their precipitation does not imply that uric acid is necessarily being passed in excessive quantity, as its solution depends upon various factors which may not be strong enough to keep a normal quantity dissolved Phosphates also pass out of solution, and are deposited when the urine is alkaline at the time it is voided, or when decomposition of urea turns the urine alkaline after it has stood for some time.

These conditions, although physiological sometimes, may be pathological at others and consequently should be carefully noted. Turbidity is always produced when blood, or pus, or fat globules are passed in quantity and these are always indicative of grave pathological conditions. Admixture with blood in large quantity makes the urine like coffee; in smaller quantity it has a smoky look. Pus, when there is much of it, gives a milky appearance, and settles down as a tenacious white deposit. Fat in the urine (lipuria and chyluria) is a rarer condition, and also makes it white, but fat does not settle like pus.

To ascertain which of these reasons is the cause of turbidity, take the reaction with litmus-papers. If it is strongly acid and the sample clears on heating, the turbidity is due to urates; this is the commonest condition. If it is alkaline, add a few drops of acetic acid, when the turbidity will clear up should it be caused by phosphates, but will persist if due to pus, blood, or chyle. Some amount of pus and blood may exist in an acid urine, but if the quantity is great these abnormal ingredients, being alkaline in themselves, render the urine alkaline. The colour is enough to tell whether turbidity is due to blood or not, and pus quickly settles and forms a viscid white deposit which becomes more viscid on the addition of potash solution. To apply this test, decant the supernatant fluid and add the solution (liq. potassæ, B.P.) direct to the suspected pus. Fat does not settle, but tends to run into globules and float. It may be shaken out with ether or carbon bisulphide.

Two or three causes of turbidity may exist together in an acid sample—there may be urates, a little blood, and a little

pus—but generally if blood or pus is present in sufficient quantity to affect the translucency, the alkalinity of either swamps the acidity of the urine. In an alkaline sample there may be phosphates, blood, and pus. A few oil globules generally indicate that a catheter has been used. The condition known as chyluria is rare.

Consistence

In health the urine is always perfectly fluid; in *diabetes mellitus*, however, a large quantity of sugar makes it less mobile, and a purulent urine may become viscid and ropy when decomposition of urea has rendered it ammoniacal. A condition known as fibrinuria sometimes occurs in profuse hæmorrhage in the urinary tract, the fibrin being derived from the blood-plasma. The fibrin coagulates into a jelly soon after the urine is voided. Fibrinuria is very rare.

Colour

The chemistry of urinary pigments is obscure, and but little understood, nevertheless much useful information may be gleaned from observation of the colour of urine. Healthy urine varies from light yellow to amber, the colour becoming darker with concentration. An acid urine is (concentration being equal) darker than an alkaline one. In disease the colour may be modified in various ways.

The consideration of the colour of urine presents some difficulty, owing to the fact that the personal element comes so much to the fore that two people, independently, seldom agree in their description of a tint. Vogel, however, suggested a very useful colour-scale, in which the colours range from pale yellow to brownish black, but its weak point is the absence of green. The table on page 7 covers the whole ground fairly well, and almost any sample can be described by one of the names. The second column is an attempt to explain the cause of the colour, and the third suggests the pathological conditions associated with such colour. Once more the reader is cautioned against too rigid interpretation of the text.

Colour.	Cause.	Condition.
Pale yellow ...	Dilution or diminution of normal pigment.	Albuminuria (various forms), anæmia, chlorosis, diabetes, hysteria.
Bright yellow ... / Yellow ...	Normal pigment.	Suggestive of no disorder.
Greenish ...	Biliary pigment.	Jaundice.
Reddish yellow / Yellowish red	Increased normal pigment (administration of certain drugs, rhubarb, senna, &c.).	Acute febrile disease.
Brownish red / Reddish brown	Blood-colouring matter, melanin, indican.	Hæmorrhage into urinary tract, melanotic sarcoma, putrefactive changes in intestine or abscess-formation.
Brownish black ...	Pyrocatechin, hydrochinone.	Carbolic poisoning, creosote, logwood.

Methylene blue and some other coal-tar colouring-matters may produce a urine tinged with the same colour.

Odour

The characteristic odour of urine is more or less pronounced according to concentration. The following distinctive smells may occur in perfectly fresh urine, and suggest pathological conditions:

Ammoniacal	=	Decomposition of urea in bladder.
New-mown hay	=	Diabetes (ferments on keeping).
Acetone	=	Grave condition of diabetes.
Sweet briar	=	Cystinuria (rare). Develops H_2S on keeping, as cystin contains sulphur.
Putrid	=	Cystitis.
Fœcal	=	Fistula communicating with intestine.

In addition, certain foods and drugs give distinctive odours to the urine, notably asparagus and garlic, turpentine (violets), sandalwood oil, and copaiba.

Froth

The froth produced on shaking normal urine quickly disappears, but is more or less permanent when bile or albumin is present. Bilious urine may have a yellow froth.

Reaction

Urine is almost always acid when voided, and the more concentrated the sample, the higher the degree of acidity. The reaction is not due to the presence of free acid, but to an acid salt—sodium dihydrogen phosphate (NaH_2PO_4). Sooner or later the urine, if it be kept, will become spontaneously alkaline from the decomposition of urea into carbonate of ammonia. The change is brought about by a ferment called *torula ureæ*, and may be represented by the equation

$$CH_4N_2O + 2H_2O = (NH_4)_2CO_3.$$

Urine voided before breakfast and during fasting is more acid than at other times; during digestion and after meals the acidity is decreased, and the reaction may even be alkaline ('alkaline side'). This is said to arise from an alteration in the blood owing to secretion of acid by the glands of the stomach. Mineral acids taken by the mouth increase the acidity, and fixed alkalies render the urine alkaline, so also do vegetable acids and salts, as they are reduced to carbonates in the system; hence fruit diet renders urine alkaline.

No information of pathological importance can be deduced from the reaction unless the variation in health and the effect of medicine be borne in mind. If that be done some useful hints can be obtained. In health increased acidity of urine is associated with increased colour; this also applies to febrile diseases, but in diabetes high acidity is found in pale urines. Again, diminished acidity is associated with diminished tissue metabolism (anæmic and cachectic states), but these conditions are parallel with diminished colour. High colour and alkalinity suggest pigmentation from drugs. The reaction is also an important index for medical treatment in various general diseases and for stone or gravel.

Litmus-papers (red and blue) are used to test the reaction, and a rough idea of the degree of acidity can be obtained by the energy of the change in colour, which may be noted as 'feebly acid,' 'acid,' or 'strongly acid.' Alkalinity may be

described in the same terms. Further information as to alkalinity may be gathered by drying the litmus-paper, when if the colour reverts to red the alkalinity is due to ammoniacal fermentation, and if the colour is permanent the alkalinity is due to earthy phosphates.

Amphoteric Urine.—Urine is seldom neutral, but it often gives both reactions with litmus-paper—hence it is described by the term 'amphoteric.' This curious phenomenon is due to the presence in solution of both acid sodium phosphate (NaH_2PO_4) and alkaline sodium phosphate (Na_2HPO_4). A good routine practice is to try the reaction with blue litmus, and if the urine is strongly acid note the sample as 'acid,' but if only feebly acid, try again with red litmus before recording, then note it as 'feebly acid' or 'amphoteric,' as the case may be. If the sample is alkaline, dry the blue litmus, and note whether this is due to volatile or fixed alkali, but remember that both volatile and fixed alkalies may exist together.

The Quantitative Determination is made by titrating with decinormal acid or alkali, using phenolphthalein as an indicator. Each c.c. of decinormal alkali (NaOH) is equal to 0·012 gramme of NaH_2PO_4, and each c.c. of decinormal acid is equal to 0·0164 gramme of Na_3PO_4. As, however, all the acid is not represented by NaH_2PO_4, seeing that free uric acid, hippuric acid, and lactic acid may be present, and as strict neutrality is hard to define, it is sufficiently accurate (for clinical purposes) to take 10 c.c. of the sample, put it into a white dish, add two or three drops of phenolphthalein solution, and run in decinormal alkali, 1 c.c. at a time, until, after stirring, a permanent pink is obtained. A good plan is to keep a small bottle of standard alkali furnished with a pinch rubber stopper pipette, graduated to 1 c.c., and no burette need be used. The small bottle can be replenished from stock as required. Instead of reporting in terms of NaH_2PO_4, the purpose of the test and guidance in treatment are just as well secured by noting the result as degrees of acidity—for, example, 10 c.c. of sample required 4 c.c. of decinormal alkali, therefore the acidity = 4°. The converse applies to alkaline urines.

Specific Gravity

The specific gravity of urine in health is as variable as the quantity; in fact, the specific gravity varies inversely with the quantity. Roughly speaking, however, the specific gravity lies between 1·015 and 1·025, but after copious drinking it may fall considerably lower, and may rise higher in exceptional concentration. In ordinary clinical work no notice is taken of temperature when determining specific gravity, but for accuracy correction should be made to 60° F. The method almost invariably adopted in urine-analysis is by hydrometer (an instrument designed for the purpose being called a urinometer), whose scale runs from 1,000 to 1,060. The urine should be poured into a cylindrical vessel of such size as will admit of the free movement of the hydrometer. Froth should be removed by brushing the surface, if necessary, with a piece of filtering-paper, and the clean and dry hydrometer is floated carefully in, the reading being taken by bringing the eye level with the lower surface of the meniscus, and observing where it cuts the stem of the instrument. If no correction for temperature is to be made, it is necessary to be careful that the sample has had time to cool to the temperature of the room; but an approximation can be arrived at by adding 1 for every 6° F. above 60° or deducting 1 for every 6° F. below 60° F. A urinometer is devised and illustrated in the woodcut, which also contains a thermometer, so that temperature and specific gravity can be read at the same

FIG. 1.—FLETCHER'S THERMO-URINOMETER.

time, and correction can be easily made as indicated above.

Solids in Urine.—The specific gravity is directly dependent upon the quantity of solids in solution, and if they were present in invariable proportion, a simple calculation would give the quantity. However, in the absence of sugar or albumin, the chief solid in solution is urea, and as it exists in very much greater quantity than any other ingredient, the variation in proportion of the rest does not seriously affect the specific gravity. An approximation can be obtained by multiplying the last two figures of the specific gravity by 2·33, thus : Specific gravity, 1·020 ; 20 × 2·33 = 46·6, which represents parts per 1,000, or 4·66 parts per cent. ; and this number multiplied by 4·375 = grains per oz. If, therefore, the specific gravity be that of the mixed and measured urine of twenty-four hours, the grains per oz. multiplied by the number of ounces will give the total solids for the day. Again, the total solids, as they consist for the most part of urea, may be taken as an index of the nitrogenous waste of the body.

Six or seven grains per lb. body-weight is about the average daily elimination of solids for a man, rather less for a woman, and more for a child. In old age the quantity is diminished. Diet and mode of life influence the solids to a great extent : a liberal eater and hard-working vigorous man consumes more material and excretes more waste than a man of opposite temperament.

In disease the total solids, and consequently the specific gravity, are altered considerably. In kidney-diseases, liver-diseases, and anæmia the total solids are diminished ; in fevers and *diabetes mellitus* the solids are increased. In *diabetes mellitus* the elimination of sugar causes the urine to become more or less dense in proportion to the quantity of sugar present, and the specific gravity may be as high, according to some authorities, as 1·075, but this is extreme. When the specific gravity is above 1·030 sugar should be suspected ; but one should never assume it is present on that account

only, nor, indeed, should its absence be taken for granted even when samples fall below 1·015.

In forming an opinion on a sample by the physical properties already enumerated, a wide view which embraces all is the only reasonable one to take. For example :—

Specimen received from a man who daily excretes 80 oz.

Characters.		Deductions.
Quantity	80 oz.	High : sugar ?
Translucency	Very clear, no deposit	Gravity should be low.
Colour	Pale yellow	
Odour	Acetone	Sugar ?
Froth	Voluminous and permanent	Albumin ?
Reaction	Strongly acid	Colour should be high.
Specific gravity	1·035	do.

Here we have pretty certainly a case of both diabetes and albuminuria, and the sample should be chemically tested accordingly. Note that no one thing points conclusively to sugar, unless it be the smell of acetone, but 'pale yellow' and 'very acid,' backed up with specific gravity 1·035, are almost certain. Again, the frothing cannot mean bile, because the colour does not agree.

Take another example :—

Characters.		Deductions.
Quantity excreted	35 oz. daily	Low.
Colour	Yellowish red	High, but gravity is correspondingly high
Translucency	Turbid, bulky pink deposit.	Urates thrown out.
Odour	Strong.	
Froth	Quickly disappears	Neither albumin nor bile suspected, but apply test for albumin.
Reaction	Strongly acid	High, so are gravity and colour.
Specific gravity	1·028	High, so is colour.

No suspicion of sugar or albumin, but both should be tested for in a routine way. The characters and deductions suggest febrile condition, but the sample is not inconsistent with exertion, with consequent perspiration, and little to drink.

Examples might be multiplied, but these are sufficient to show that in forming an opinion upon the physical characters no isolated one is, as a rule, sufficient in itself, but the interdependence of one upon another must be considered before judgment can be formed. Then only a tentative verdict can be passed ; recourse must be had to chemical analysis, supported perhaps by microscopic examination.

Familiarity with, and a proper appreciation of, the abnormal physical characters of urine are very important, for it is often in consequence of them alone that chemical analysis is resorted to, and they generally indicate the line along which the investigation of any sample should be conducted. It is well also for the reader to remember that sophistication, which is by no means uncommon, as a rule aims at altering the naked-eye appearance, colouring-matter being a favourite addition. Again, accidental admixture by altering the appearance causes anxiety and often drives a nervous person to seek advice, although his health may be good. Another thing to bear in mind is the fact that some of the physical characters may alter spontaneously. For example, a specimen that is clear when passed may become turbid on cooling or on keeping. The colour also sometimes changes, notably in carbolic-acid poisoning, when the sample by exposure darkens very appreciably.

CHEMICAL ANALYSIS OF URINE.

THE chemical processes involved in testing the reaction have already been considered in the previous chapter. This chapter deals with the chemical tests qualitative and quantitative, first of the normal ingredients of pathological importance, and then the abnormal ones. But there are a few points to be observed in the preliminary handling of the specimen. A few conical glasses of about 6 oz. capacity should be available, into one or more of which the specimens should be poured, and the physical characters noted. If the glass be now set aside for a few hours the insoluble matter will separate, generally settling down to the bottom, although it occasionally floats. This practice has many advantages. It generally secures a clear supernatant fluid without filtering (an essential for some of the tests), and gives a better idea of the cause of the turbidity, while it collects the material for the microscopical examination.

The normal nitrogenous constituents that require most attention in this book are urea and uric acid and urates. Xanthine, creatinine, and hippuric acid demand but brief notice.

Urea

Urea or Carbamide [$CO(NH_2)_2$ or CH_4N_2O] is by far the most important nitrogenous waste product in urine, and exists in much larger proportion than any other; in fact, urea accounts for about 90 per cent. of the total nitrogen in urine.

The quantity of urea per diem excreted by a man averages about 500 grains, but this, as will be seen later, varies considerably. Urea has a classical place in chemical literature, as by the synthetic production of it in 1828, Liebig and Wöhler broke down the barrier between organic and inorganic chemistry. It is an extremely soluble substance, hence it is invariably found in solution in urine. It is very stable, and may be separated and purified, when it forms crystals such as those depicted in the figure, colourless, odourless, and of saline taste. Its solution has no alkaline reaction to litmus, yet it possesses strong basic properties, readily combining with acids to form salts of well-defined crystalline form (*see* figures of urea nitrate and urea oxalate).

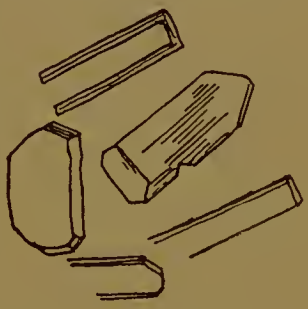

FIG. 2.—UREA CRYSTALS.

FIG. 3.—UREA NITRATE.

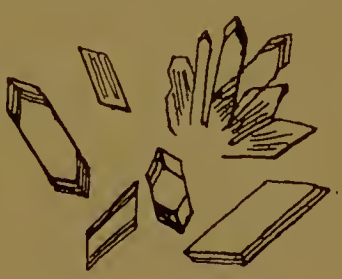

FIG. 4.—UREA OXALATE.

Although urea is so stable, it readily falls a prey to fermentation when in combination with other organic matter such as exists in urine. The ferment is known as *torula ureæ*, and under its influence urea undergoes hydrolysis, as represented by the following equation:

$$CO(NH_2)_2 + 2H_2O = CO_3(NH_4)_2.$$

The resulting compound is carbonate of ammonia, and hence the urine becomes alkaline and has the odour of ammonia, so the change is commonly called 'ammoniacal

fermentation.' This process may go on in the bladder when the torula is introduced by the use of a catheter which has not been made thoroughly aseptic.

A qualitative test for urea is seldom required. It may be performed by taking advantage of the salt-forming properties of this substance. Evaporate a small quantity of the suspected fluid to about a fourth of its volume, place a drop or two on a microscope-slide, add a drop of strong nitric acid, and observe under a low power. If urea is present, rhombic or hexagonal plates of urea nitrate will be formed (fig. 3).

The quantitative determination of urea is, on the contrary, of first-class importance. An approximate estimate in urines free from sugar and albumin may be made from the specific gravity.

It has already been stated that urea is the most plentiful constituent of urine, and chiefly governs the specific gravity; consequently, specific gravity is an index of the approximate quantity of urea. By dividing the last two figures of the specific gravity by 10 the urea is expressed in percentage, thus :

$$\text{Specific gravity } 1{\cdot}025\ ;\ \frac{25}{10} = 2{\cdot}5 \text{ per cent.}$$

Fortunately, however, a much more accurate chemical estimation can be accomplished with very little trouble. The process in general use is known as the hypobromite method, and it depends upon the fact that when urea is acted upon by sodium hypobromite it is decomposed, with formation of carbonic-acid gas and nitrogen. The hypobromite solution is made with excess of caustic soda, so that the carbonic-acid gas combines with it as fast as it is formed, and only the nitrogen escapes as gas. The reaction is represented by equation thus :—

$$CO(NH_2)_2 + 3NaBrO = 3NaBr + 2H_2O + CO_2 + N_2.$$

It will be seen, therefore, that by conducting the reaction in a suitable apparatus so that the evolved nitrogen can be

measured, data for calculating the quantity of urea will be obtained.

Hypobromite solution is best used fresh, as it does not keep long. It is easily made as follows :

Dissolve 100 grammes ($3\frac{1}{2}$ oz.) of caustic soda in 250 c.c. (9 oz.) of water. Label this 'Soda Solution for Hypobromite.' To this solution bromine must be added, gradually, at the time it is wanted in proportion of 1 of bromine to 10 or 12 of the soda solution. A convenient way of mixing is to pour out the caustic-soda solution in a conical measure—℥j. if 1 c.c. and ℥iv. if 5 c.c. of urine is to be used—then, without emptying, add the bromine, which sinks through the soda, and can be measured under it ; now gradually stir until the two unite to form the sodium-hypobromite solution.

Many forms of apparatus have been devised for the estimation. The simplest is that made by Southall, and designed by Dr. Doremus, New York. It consists (1) of a bent graduated tube closed at one end, and with globular expansion at the other, mounted upon a wooden stand in an inverted position, and (2) a pipette with one graduation for measuring 1 c.c. of the sample to be tested. It is used as follows : Hypobromite solution is poured into the apparatus until it reaches a certain mark near the bend (about 5 c.c. is required). Water is next added carefully so as not to mix more than can be helped with the denser hypobromite solution, and in sufficient quantity to nearly fill the bulbous portion of the tube. Now incline the apparatus in such a way that the water shall flow into the long limb and replace the air, which will bubble out and escape by the bulb. Erect the apparatus again, and if the operation has

FIG. 5.—SOUTHALL'S UREOMETER.

been done carefully the long limb will be full of water and the hypobromite will be found undiluted in its original place at the bottom of the bend. Draw urine into the pipette up to the 1 c.c. mark by means of the teat and inject it into the hypobromite solution, taking care that the tip is introduced well under the long limb, else some of the gas will be lost. The nitrogen is

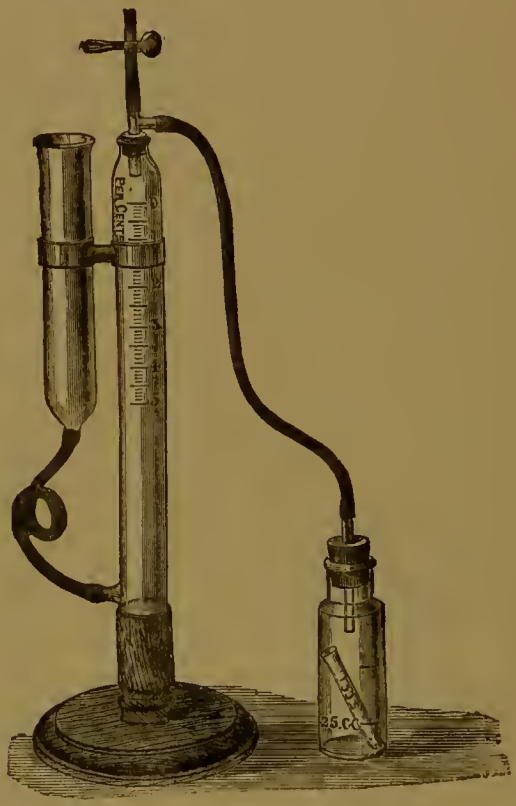

FIG. 6.—GERRARD'S UREOMETER.

soon all evolved and collects in the graduated limb. After the froth has subsided it may be read off. The graduations are so arranged that a calculation is unnecessary, as each division indicates 0·001 gramme of urea in 1 c.c. of urine taken, and by multiplying the result by 100 the percentage is obtained, or by 437·5 the number of grains per oz.

An improvement upon this form of apparatus is called, after its inventor, Gerrard's ureometer (*see* fig. 6). It is used as follows: Pour into a test-tube 5 c.c. of urine, and into the bottle 25 c.c. of sodium-hypobromite solution; place the test-tube inside the bottle, taking care not to spill any of the contents. Fill the graduated tube with water so that the level reaches the zero line. This is done by removing the clip at the top and pouring water into the side tube, which should be placed high, so that little water remains in it. Now connect the indiarubber tubing to the bottle, and note that the water is at zero. Close the clip. Upset

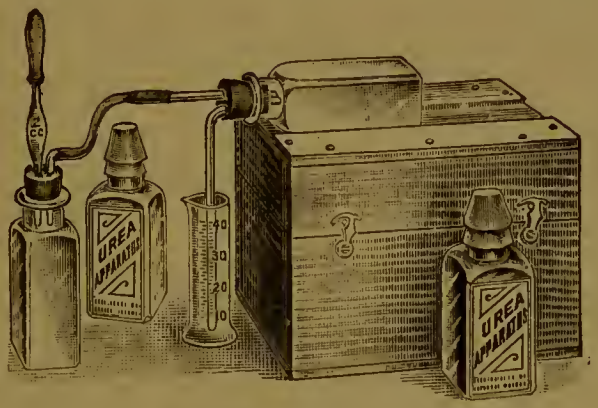

FIG. 7.—MARTINDALE'S UREA-APPARATUS.

the contents of the test-tube into the hypobromite solution; nitrogen is evolved, and pushes back the water into the side tube. When this ceases, lower the side tube until the levels of the water in both tubes are equal; then note the volume of gas, which shows the percentage of urea. The solution of hypobromite of soda recommended by Gerrard is made by dissolving 100 grammes of caustic soda in 250 c.c. of water and adding 22 c.c. of bromine.

Another method is illustrated in the above engraving of Martindale's urea-apparatus. Here the urine and reagents are placed in the bottle to the left, and the evolved nitrogen bubbles into the bottle lying on the case, with the result that the water in the bottle is displaced by the gas, and the volume

of water displaced is noted. Tables are supplied giving at a glance the content of urea per cent., the number of grains per fluid oz. and per pint.

When a nitrometer is at hand, special apparatus need not be obtained. If the nitrometer is fitted with a three-way tap, the tap can be attached to the generating-bottle, as in Gerrard's instrument; but if not, the urine must be measured into the cup, and then run cautiously into the nitrometer itself. The cup must next be rinsed with a little water, and that also run into the nitrometer; then measure the hypobromite into the cup, and run that in, taking care that no air follows and that no gas escapes. A vigorous shake will be necessary before the reaction is complete. The nitrogen in this case is measured in c.c.'s, and a calculation is necessary to convert it into terms of urea. Thus, 37·1 c.c. of nitrogen may be taken to represent 0·1 gramme of urea; therefore if x = no. of c.c. found, $\frac{x \times 0{\cdot}1}{37{\cdot}1}$ = no. of grammes of urea in the 5 c.c. of urine taken, and that multiplied by 20 = percentage; or the fraction may be reduced to the single factor 0·054, and $x \times 0{\cdot}054$ = percentage of urea, or $x \times 0{\cdot}236$ ($0{\cdot}054 \times 4{\cdot}375$) = gr. per fl. oz.

No correction is required for temperature, barometric pressure, or tension of aqueous vapour, as these just about compensate for the fact that about 8 per cent. of the nitrogen of the urea is not given off as gas. If, however, the sample contain much sugar, a correction is required in the reading of all the instruments, as instead of only 92 per cent. being given off, 99 per cent. of nitrogen is set free. In cases of diabetes, therefore, a truer estimate of the urea is made by multiplying the figures obtained by $\frac{92}{99}$ or 0·93. Of course if refinement of analysis is required, all these corrections for pressure, temperature, and tension of aqueous vapour must be rigorously carried out. For ordinary clinical work, however, the result is sufficiently near the mark without the trouble of the calculations.

Increase of Urea.—About 2 per cent. is the average quantity of urea in urine, but this quantity may be increased

directly with the quantity of animal food and exertion, and inversely with the volume of urine passed. A pathological increase takes place in fevers and diabetes, also in poisoning by phosphorus or arsenic.

Decrease of urea takes place physiologically in diminished diet and sedentary habits. A pathological decrease takes place in certain liver-diseases, in anæmia and other debilitating diseases, and particularly in kidney-diseases. It is in the pathological cases that urea-determinations are most important. In the former less urea is created in the body; while in kidney-diseases the urea is formed, but not eliminated, and, increasing in the system together with other effete matter, has a toxic effect known as uræmic poisoning, which, if not rapidly relieved, ends in death.

Uric Acid

Uric Acid ($H_2C_5H_2N_4O_3$) is the second most important nitrogenous waste product found in urine. It has received a great deal of attention from chemists, and the reader is referred to works on pure chemistry or to larger books on urine for elaborate accounts of its chemistry. It is a dibasic acid, and generally exists in urine combined as alkaline urates. By abbreviating the uric-acid formula to '$\bar{U}$,' the two salts would be represented as $M_2\bar{U}$ and $M\bar{H}\bar{U}$. The neutral urate is a laboratory salt; the biurate is sometimes found in urine, but a third salt, known as a quadriurate, appears to be the one that exists normally in urine. This is represented by the formula $M\bar{H}\bar{U},H_2\bar{U}$, one molecule of biurate being combined with one molecule of uric acid. It is this salt which so often separates from highly acid urines and forms the so-called brick-dust deposit. Besides existing as salts, uric acid is sometimes found free in the urine, and, being very slightly soluble, it separates in crystals often visible to the naked eye like Cayenne pepper. A further notice of the crystalline forms will be found in the microscopical section.

Qualitative Test for Uric Acid and Urates. — Evaporate a little of the suspected sample to a small bulk in a

porcelain dish, add a drop or two of strong nitric acid, and continue the evaporation to dryness on a water-bath. A yellowish-red residue is left. Now invert the dish over an open bottle of ammonia solution so that the fumes shall act upon the residue, and a purplish-red colour will result. This is known as the murexia test, from *murex*, a kind of whelk from which the ancients extracted a similar colour.

Quantitative Determination.—The old method of precipitating by means of hydrochloric acid was found to yield too low results, and consequently it has been abandoned in favour of a process which depends upon the insolubility of acid ammonium urate in a solution of ammonium chloride. It is conducted as follows : Shake up the sample so as to include any precipitated uric acid ; take 100 c.c. of it, add 30 grammes of ammonium chloride and stir every few minutes until solution is effected. This generally does not take place until the depression of temperature begins to recover. Next add a few drops of ammonia until the solution is rendered alkaline, and allow to stand for ten minutes. By this time the acid ammonium urate will have settled and may be separated by filtration. Wash the separated salt upon the filter with a saturated solution of ammonium sulphate. When thoroughly washed and all the sulphate has passed through, punch a hole through the point of the filter-paper and wash the precipitate through with a jet of hot water into a clean beaker. Cool and make up to 100 c.c. Add 20 c.c. of strong sulphuric acid to acidify the liquid and render it hot, and whilst it is hot titrate with a $\frac{N}{20}$ solution of permanganate of potash (1·578 gramme per litre) until a pink colour is formed which survives agitation and lasts some seconds. Each c.c. of permanganate represents 0·00375 gramme of uric acid.

The weak point in the process lies in the end-reaction, as the appearance of the pink colour is neither sharp nor stable. It is not much good to try to work to greater nicety than a c.c. at a time of the permanganate solution, and this gives results sufficiently accurate for clinical purposes. Estimated by this

method, the average quantity of uric acid excreted by a full-grown man in health amounts to about 1 gramme or 15 gr. per day; but this figure is liable to considerable fluctuation without implying any departure from health.

Pathologically, uric acid is increased in fevers and in diseases resulting in nuclein-destruction, such as leucocythemia. It is diminished in gout and rheumatism, presumably on account of the soluble quadriurate being changed in the blood to the insoluble biurate, and thus it is retained in the system instead of being eliminated.

Other Nitrogenous Constituents

Xanthine Bases are closely allied to uric acid, but no satisfactory method for estimating them has been devised, nor, indeed, has their physiological or pathological significance been established. The reader is referred to larger works for more detailed information.

Creatinine is a constant ingredient of urine, but as the variations in its quantity have no recognised clinical importance, it is merely mentioned here as a nitrogenous waste product. One or two of its properties must not be overlooked, however, for it reduces Fehling's solution and produces a deep-red colour with picric acid and alkali.

Hippuric Acid is excreted in quantities varying from 7 to 15 gr. or more per diem, chiefly as hippurates of soda and potash. It is increased by eating much fruit, especially plums, blackberries, and cranberries—fruits which contain aromatic acids. The administration of benzoic acid also increases the elimination of hippuric acid. It is found in much greater quantity in the urine of herbivorous animals. Its clinical significance is doubtful, and consequently it does not demand much consideration in this book.

A determination of some consequence is the total nitrogen, which includes all the foregoing. It is best done by Kjeldahl's method, modified by Allen as follows :—

Take 25 c.c. of urine, add 10 c.c. of strong H_2SO_4, boil gently in a porcelain basin until reduced to about 10 c.c. and white fumes of sulphuric acid are evolved. The liquid is then allowed to cool and carefully

transferred to a pear-shaped flask, the basin being rinsed with a few drops of water. The flask is placed in an inclined position, to prevent loss by spurting, and the contents kept in gentle ebullition. If excessive frothing occurs, it may be moderated by adding a small fragment of paraffin wax (candle). When the frothing has ceased, about 5 grammes of potassium sulphate should be added, and the flask heated strongly until the liquid is colourless or only a very pale yellow. The contents of the flask are then allowed to become cold, when about 20 c.c. of water is added very cautiously, and a few drops at a time, agitating the liquid by a rotatory movement after each fresh addition. A highly concentrated solution of caustic soda, made by dissolving the alkali in about an equal weight of water, is now added gradually, with constant agitation, until the sulphuric acid is nearly neutralised. This point may be ascertained by means of litmus-paper, or a few drops of litmus or phenolphthalein solution may be added to the contents of the flask.

The neutralised liquid, which will measure about 80 c.c., is now diluted to exactly 100 c.c. with water, and thoroughly mixed by agitation. Ten c.c. of the solution, representing 2·5 c.c. of the original urine, is now treated with 10 c.c. of the alkaline hypobromite reagent used for the determination of urea, and the manipulation is the same as is described in the section on urea.

The volume of 24 c.c. at ordinary pressure and temperature represents 0·028 gramme of nitrogen, therefore to calculate the percentage result the following equation can be used:

$$N = \frac{G \times 0{\cdot}028 \times 100}{U \times 24}$$

where G = gas obtained and U = urine used—namely, 2·5 c.c. The whole reduced to a factor is $N = G \times {\cdot}046$.

If urea and uric acid have been separately determined, the quantity of nitrogen they represent can be deduced from their formulæ, and the sum of the two, subtracted from the total nitrogen determined as above, will leave nitrogen obtained from hippuric acid, creatinine, &c.

Non-Nitrogenous Constituents.

Phosphates are found in the urine as salts of sodium, potassium, calcium, and magnesium. The combinations of phosphoric acid with the former two bases constitute the alkaline phosphates, and with the latter two the earthy phosphates. Earthy phosphates are precipitated in the urine when it is rendered alkaline or merely neutral, and under such conditions may be deposited before it leaves the bladder. This may arise by

the use of alkalies as therapeutic agents, or by the ammoniacal fermentation. When urea has undergone this fermentation, either in the body or after the urine has been passed, an additional earthy phosphate occurs—viz., the ammonio-magnesium phosphate; this salt must therefore not be looked upon as a normal one, but as a product of decomposition. Earthy phosphates are said to represent three-fourths of the total phosphates eliminated, and the sodium salt is in greater proportion than the corresponding potash one. The earthy phosphates are insoluble in water, but they readily dissolve in an acid medium. The alkaline phosphates are readily soluble, but this name is misleading—it is more correct to say phosphates of the alkali metals, for they exist as acid salts; in fact, it is the acid phosphates of sodium and potassium that render the urine acid and also keep the earthy phosphates in solution. There is, however, another factor in the solution of the earthy phosphates, as is evident sometimes on boiling a sample. The heat drives off carbonic-acid gas, and a cloud of phosphates is deposited. This cloud may be mistaken for albumin, but any doubt, like the cloud, is cleared up by a drop or two of acetic acid, which immediately redissolves the phosphates. The separation of phosphates from solution would not occur in a distinctly acid sample.

Qualitative Test.—Add ammonia, and earthy phosphates are precipitated; filter and add magnesia mixture to the filtrate, and alkali phosphates are precipitated.

Quantitative Test.—This is best done by titrating with standard solution of uranium nitrate, using a solution of potassium ferrocyanide as indicator. The solutions required are:

A standard solution of uranium nitrate, of which 50 c.c corresponds exactly to 50 c.c. of standard phosphate solution, each c.c. of uranium solution representing 0·01 gramme of P_2O_5. (*See* page 77.)

To a solution containing 100 grammes of acetate of sodium dissolved in water, 100 c.c. acetic acid is added, and the mixture made up to 1,000 c.c. (*See* page 77.)

A weak solution of ferrocyanide of potassium.

Of the filtered urine 50 c.c. is mixed in a beaker with 5 c.c. of the acetate solution, the beaker is put on a water-bath, and when the mixture is well warmed add uranium solution until the mixture ceases to give a precipitate, which can be ascertained by allowing the solution to trickle down the side of the beaker. A drop from the beaker is placed on a porcelain dish with a glass rod, and a drop of ferrocyanide solution placed near it with another glass rod, and the two drops are allowed to run together. If no alteration of colour be produced at their union, more uranium solution must be added to the urine, until the drops, when meeting, produce a light-brown colour. The number of c.c. of uranium solution used is read off, and if after a second warming of the urine in the beaker, and a second trial of the fluid with the ferrocyanide, no increase in the intensity of the colour be produced, the process may be regarded as complete; but if a dark-brown colour be formed at the second trial, too much uranium solution has been added, and the estimation must be started again with another 50 c.c. of urine.

To calculate the amount of P_2O_5 passed in twenty-four hours: If 1 c.c. of uranium solution represents 0·01 gramme of P_2O_5, and if 15 c.c. of uranium solution has been used, 50 c.c. of urine will contain $0{\cdot}01 \times 15 = x$ gramme of P_2O_5; and if the patient has passed 1,500 c.c. of urine in twenty-four hours, then the calculation will be

$$50 : 1{,}500 :: 15 \times 0{\cdot}01 : x \text{ grammes.}$$

The quantity of phosphoric acid eliminated daily by a healthy adult living on a mixed diet varies from 2 to 3 grammes. In renal diseases phosphates are diminished proportionately with the other urinary ingredients. A diminution has also been observed at the beginning of fevers, but an increase occurs as the fever subsides. Increase is said to occur in wasting diseases of the nervous system. But the chief importance of the recognition of phosphates does not lie in the quantity eliminated (as no very useful information can be based upon it) but upon the fact of phosphates falling out of solution. The condition that governs the

solution of phosphates is acidity: if this fails the earthy phosphates are thrown out. Certain cerebral and nervous lesions produce this condition, and persistent alkaline and phosphatic urine with no medicinal or dietetic cause should direct attention to the nervous system. Again, phosphatic urine may produce stone, because the deposit of phosphates in the urinary system soon aggregates round any nucleus that may exist, such as epithelial cells.

Chlorides exist in urine as sodium and potassium salts, chiefly sodium chloride. Of the inorganic constituents, chlorides are present in the greatest proportion. The physical properties of sodium chloride need no description.

Qualitative Test.—To a small quantity of urine in a test-tube add excess of nitric acid and solution of nitrate of silver: a white curdy precipitate is formed.

Quantitative Test.—This is best done by adding excess of standard solution of silver nitrate to a measured quantity of urine previously acidified with strong nitric acid, and titrating the excess of silver with standard ammonium sulphocyanide.

Process.—Take 10 c.c. of the sample and dilute to 50 c.c., add 2 or 3 c.c. of strong nitric acid and 20 c.c. of decinormal nitrate-of-silver solution. This precipitates the whole of the chlorides, while phosphates, &c., and excess of silver remain in solution. The excess of silver nitrate is now ascertained by titrating with decinormal ammonium sulphocyanate, using a few drops of strong solution of ferric ammonium sulphate as the indicator.

The titration must be conducted with constant stirring, until a permanent colour of ferric sulphocyanate is formed. (It is necessary to check the sulphocyanide against the decinormal $AgNO_3$ from time to time, and adjust its strength until it corresponds c.c. for c.c.) The number of c.c. of sulphocyanide required, deducted from the 20 c.c. of silver solution, indicates the number of c.c. of silver solution acted upon by the chlorides, thus—

$$AgNO_3 + NaCl = AgCl + NaNO_3.$$

10	168·69	58·07
	16·869	5·807

Therefore each c.c. of $\frac{N}{10}$ $AgNO_3$ used represents 0·0058 gramme of NaCl, and the calculation from 10 c.c. to parts per 1,000, or grains per oz., or for the day, is simple.

Chlorides always exist in considerable quantity in health, but the absolute quantity varies within very wide limits, being governed largely by diet. In fevers, however, the output of chlorides is greatly diminished, particularly in pneumonia, in some cases of which, indeed, it is almost suppressed. An estimation may therefore be the means of clearing up a doubtful case.

Sulphates exist in the urine in two forms—viz., inorganic sulphates and sulphates of organic radicles. The inorganic sulphates are probably potassium and sodium salts, while the organic sulphates (or ethereal salts) are probably salts of phenyl sulphuric acid, indoxyl sulphuric acid (*see* indican), skatoxyl sulphuric acid, and allied sulphates. The inorganic salts are ten times more plentiful than the ethereal salts. The sulphates, like the chlorides, are always in solution. Besides the sulphates, other sulphur-containing substances exist in urine, notably cystin.

Qualitative Test. — Acidify strongly with hydrochloric acid and add barium-chloride solution, and a white, heavy precipitate of barium sulphate will fall. To test for ethereal salts, first separate the metallic salts as follows : Acidify with acetic acid, add barium-chloride solution, and the metallic salts are precipitated ; filter, and to the filtrate add strong hydrochloric acid and boil. This breaks down the ethereal salts, and the sulphates are precipitated as barium sulphate.

Quantitative Test.—A rough idea of the quantity of sulphates may be ascertained by the qualitative test, the bulk of the precipitate being the guide ; but accurate determination can only be obtained by weighing the precipitates. Perhaps the best procedure is to make two determinations on two separate quantities of the sample, the first being the total sulphates, and the second the ethereal sulphates ; then, by deducting the latter from the former, the metallic sulphutes are found.

Take 100 c.c. (or 50 will do) of urine, add 5 c.c. of strong hydrochloric acid, and boil for fifteen minutes, then add 10 c.c. of B.P. barium-chloride solution, and boil again for a few minutes. Allow the precipitate of $BaSO_4$ to settle, and decant the supernatant fluid through a filter of known ash value, retaining the precipitate as much as possible in the beaker. Wash the precipitate by decantation with two or three quantities of boiling water, and finally transfer the precipitate to the filter. Wash the filter and precipitate with hot methylated spirit (non-mineralised), and lastly with ether. Ignite the filter in a tared platinum or porcelain crucible, or, better, wrap the filter round with platinum wire, and ignite it over the crucible, and shake the ash into the crucible ; add a drop of sulphuric acid, and raise to red heat. Cool under a desiccator. Weigh. Deduct the tare of the crucible and filter-ash, and the result gives $BaSO_4$ from 100 c.c. of urine. To express the result as SO_3 multiply this number by $\frac{80(SO_3)}{233(BaSO_4)}$, or, expressed as a decimal, 0·34335. This again multiplied by the number of hundreds of c.c. of urine passed in the twenty-four hours will give the total daily elimination of sulphates.

For organic sulphates first precipitate the metallic sulphates with alkaline barium chloride.

Take 100 c.c. (or more rather than less) of urine, add 20 c.c. of baryta-water and 10 c.c. of barium-chloride solution. In a few minutes all the metallic sulphates and phosphates will precipitate. Filter off 65 c.c., representing 50 c.c. of urine, add 10 c.c. of strong hydrochloric acid, and boil. Maintain the boiling temperature for about an hour, preferably on a water-bath, as it is prone to bump, and set aside for a day for the $BaSO_4$ to settle. Filter, wash, and ignite the precipitate as in the process for total sulphates, and calculate the SO_3 by the same formula.

The boiling with hydrochloric acid breaks down the organic combinations, and admits of the precipitation of the sulphuric acid as barium sulphate. The calculation is the same as before, and the result represents SO_3 in combination with ethereal bases. The quantity of sulphates depends largely upon the kind of food consumed, but it may be said roughly to be about 2 grammes of SO_3 per diem, about one-tenth of which is represented by the ethereal sulphates. These latter are increased by putrefactive changes of foodstuffs in the bowel or by abscesses.

Oxalates.—Oxalate of lime is frequently found in the microscopical examination of urines (which *see*), as it is an extremely insoluble salt. It is said to be held in solution to some extent by the acid phosphate of sodium of the urine. No simple qualitative test can be applied for its detection in solution, as only about 1 gr. is eliminated in the whole twenty-

four hours, and the quantitative test is cumbersome and of no recognised pathological importance. A micro-chemical qualitative test is, however, useful in examining a deposit, and it should be performed upon the slide and observed under the microscope: oxalate-of-lime crystals are insoluble in acetic acid, but readily dissolve in mineral acids.

ANALYSIS OF ABNORMAL CONSTITUENTS

So far, chemical analysis has dealt solely with those constituents of the human urine whose presence is consistent with health, although the variation in amount may indicate disease and determination of the quantity assist the physician in diagnosis or treatment. The chemical properties and testing of abnormal constituents are considered in this chapter.

Albumin

Albumin (spelt also albumen by some writers, but the latter spelling should be confined to the albumen of eggs) is frequently found in human urine. Difference of opinion exists amongst authorities as to the significance of a trace of albumin, but all admit that its presence is a danger-signal which should never be disregarded, and if albumin exist in any quantity it is always of grave pathological importance. In urinary analysis, the term 'albumin' generally covers all the proteids that may exist in urine that coagulate upon boiling or with nitric acid. Besides these, there are other proteid bodies known, such as albuminates, albumoses, and peptones, which will receive brief notice later on. Albumin generally consists of serum albumin and paraglobulin in varying proportion, but as the separation and estimation of the two substances involve a considerable amount of time and trouble, and as, moreover, no pathological information is gained by the knowledge, it is customary to test for the two combined, and in estimation to treat the two proteids as one substance—albumin.

In testing for albumin in urine many different methods may be adopted, but all depend upon rendering the albumin insoluble, and therefore evident either as a precipitate, or as a cloud, or as a very slight haze according to the quantity

present. It will, therefore, be easily understood that it is essential, especially in the case of traces, that the urine and the testing-solution should be perfectly clear. To obtain urine clear is not always easy, particularly if it be a little stale and bacteria are plentiful in it. If simple filtration will not effect it, add a few drops of potash solution (B.P.), which will precipitate the phosphates, and these carry down the bacteria. The clear filtrate must then be acidified again with acetic acid until it sharply reddens litmus-paper. The urine is then ready for the application of the following tests.

Heat Test.—This, perhaps, is both the simplest and most satisfactory test, and, with a little practice, as sensitive as any. Take a long test-tube, half fill it with the filtered and, if necessary, acidulated sample (if the urine is sharply acid to litmus no acidulation is necessary) and boil the upper half of the column by holding it slanting in a Bunsen or spirit-lamp flame. If albumin is present, it will coagulate with the heat and form a white precipitate, more or less dense according to the quantity. If there is much, it is easily seen ; but if only a small quantity is present, it will be necessary to examine it with some care. Face the window and look at the tube with a black background, such as the back of this book or your coat-sleeve, holding the tube an inch or two away from it. The reason for only boiling the upper half of the column will now be seen, as the lower unboiled half makes a contrast with the upper boiled half, and the merest trace of coagulated albumin can be detected by comparison. Another way is to have the urine in two similar test-tubes, to boil one and keep the other for comparison. The coagulum or cloud that forms should always be verified, as often there is a precipitate of earthy phosphates produced by boiling which may easily be mistaken for albumin. The distinction is easily made by dropping one or two drops of nitric acid upon the interior side of the tube and allowing it to run slowly down into the urine, when phosphates are immediately dissolved while albumin remains insoluble. A word of caution, however, is necessary in using nitric acid : add it very cautiously—one drop is generally

sufficient—or an acid albuminate which is soluble may be formed. If this is suspected, continue the addition of the acid more liberally, and the albumin will again pass out of solution. Some chemists prefer to use acetic acid to avoid this risk.

Heller's Test.—Pour strong nitric acid into a test-tube to the depth of about half an inch ; then gently run about an inch of the urine upon the top of it so as to disturb the nitric acid as little as possible. This is conveniently done with a pipette. In the presence of albumin a white line is formed at the junction of the two liquids. There are several drawbacks to this test, owing to the action of the nitric acid upon other urinary constituents, which mask the effect to some extent. The urea, in a concentrated urine, may combine with the nitric acid and form crystals of urea nitrate. Normal colouring-matter is darkened considerably by nitric acid, and bile pigment, if present, produces the play of colours characteristic of bile (*see* Bile, page 50). Albumose or peptone also produces a ring, which, however, disappears on heating and reappears on cooling. If the patient is taking resinous drugs, like copaiba, there may be sufficient resin in the urine to be precipitated by the acid. Such a precipitate will dissolve in alcohol. Drugs of this character generally announce themselves by their odour.

Picric-acid Test.—A saturated solution of picric acid (gr. vj. to ʒj.) coagulates albumin in acid solution. To about an inch of clear urine (acidulated if necessary) in a test-tube add a like quantity of picric-acid solution ; in presence of albumin a coagulum is formed, varying from heavy flakes to the lightest cloud, according to the quantity of albumin. This test is extremely delicate, but the operator must bear in mind that picric acid precipitates other proteids as well as albumin ; therefore, if the latter body is suspected, it is necessary to warm the test-tube, when a precipitate due to other proteids will dissolve, while an albumin precipitate will remain unaltered. The presence of drugs, such as quinine and other alkaloids and piperazine, also produces a precipitate, which, however, dissolves on heating.

Ferrocyanide Test.—A solution of potassium ferrocyanide coagulates albumin in acid solution. Fill a test-tube to the depth of 2 inches with clear urine and acidulate it strongly with acetic acid (about 10 ♏) ; then add, drop by drop, potassium-ferrocyanide solution, B.P. In the presence of even traces of albumin a white cloud will form round the drops as they fall into the urine. If, upon the addition of the acetic acid only, a white cloud forms, it is probably due to mucin, and the sample must then be filtered before the ferrocyanide is added.

Besides the foregoing tests there are a host of others more or less sensitive and useful, but it is unnecessary to burden a small book with an account of them. If one of the four tests already described gives a reaction about which there is any misgiving, it can generally be cleared up by applying one or more of the other three. If they are intelligently applied, the merest trace of albumin should be made evident by all of them, and therefore, if one fails, the analyst should look for something else as the cause of the reaction in the other three. As to the respective sensitiveness of the tests there is not much to choose, but the heat test is the fail-me-never of experienced practitioners.

Quantitative Test. — An absolute quantitative test for albumin is not available for clinical use—first, because of the difficulty of drying and weighing albumin, and, secondly, because, as already stated, albumin is not a single substance, nor a mixture in definite proportions. However, an approximate quantitative test, and one which satisfies the clinical demand, is a very simple matter. It is obtained by roughly measuring the bulk of coagulum. The heat test used to be the standard, and the albumin used to be read off as a fraction of the height of the column of urine in the tube (the whole length of course was boiled), and reported as $\frac{1}{10}$ or $\frac{1}{2}$, &c., as the case might be. But this plan is now superseded by Esbach's method, which depends upon the bulk of coagulum produced by picric acid.

The estimation is made in an instrument known as

Esbach's albuminometer, which consists of a long and narrow graduated test-tube fixed on a stand. Urine is poured into the tube up to the mark U; the reagent (10 grammes of picric acid and 20 grammes of citric acid in a litre of distilled water) is added up to the mark R. The tube is then closed by the thumb, and the liquids are mixed by turning it upside down several times; shaking should be avoided. The tube is then allowed to stand upright for twenty-four hours, and the mean height of the coagulum read off. The figure at which the level stands indicates the amount of albumin in grammes per 1,000 c.c.

FIG. 8.—ESBACH'S ALBUMINOMETER.

When the albumin is very abundant, the urine may be diluted with an equal quantity of water and the result multiplied by 2.

The citric acid is added to the picric-acid solution in order to make it strongly acid and ensure the coagulation of the albumin.

The pathological importance of detecting albumin in the urine cannot be over-estimated. Although some authorities consider that a trace may not be inconsistent with health, on the other hand a trace may be evidence of the gravest disorders in incipient stages. The duty of the analyst is therefore perfectly plain: he should exert every effort to detect the slightest trace. The causes of albumin in the urine are numerous. In the kidneys the blood-circulatory system is in the closest possible relation to the urinary system, and the effete matter of the former filters through into the latter; but if any disturbance of the pressure of the blood causes increased force to bear upon the intervening cells, not only the effete matter, but also the albuminous matter of the blood filters through.

Certain diseases of the heart are notable examples of albuminuria of this class.

Again, the albuminous matter of the blood may appear in the urine in consequence of faults in the kidneys themselves by which they fail in their selective power through morphological changes in their cells, as is the case in the maladies known under the generic name of Bright's disease, and also in those fevers (notably scarlet fever) the poisons of which induce acute nephritis. Hæmorrhage into the urinary system, which may arise in many localities from a great variety of causes, is another source of albuminuria, as also is the inflammatory process resulting in pus-formation, notably in the pelvis of the kidney and in the bladder. In ascertaining the cause or source of the albumin, much may be done with the microscope (*see* microscopic section).

Albumose.—Besides the proteids classed under 'albumin,' others may exist in the urine. The allied bodies known as albumoses or proteoses are sometimes found, and so is peptone. The chemistry of these substances is somewhat obscure. The albumoses or proteoses are intermediate bodies between albumin and peptone. They are not coagulable by heat, but are precipitated in the cold by acids. This precipitate is, however, redissolved by heat. They also produce a rose-red colour with Fehling's solution, but a better test is to add *one* drop of the copper solution of Fehling's reagent to a test-tube in which urine has been poured to the depth of an inch, and then mix half as much potash solution with it, when the presence of albumose produces a rose-red colour (biuret reaction). Peptones are neither coagulable by heat nor precipitable by acids, but they give the biuret reaction.

Urines in which albumose or peptone is present (probably a mixture of the two in many cases) commonly indicate pneumonia, but they are also a symptom of empyema. The rare condition known as *mollites ossium* is also said to produce albumosuria or peptonuria. If albumin is present, it must be removed previous to testing for other proteids. This is best done by coagulating the former by heat, filtering while still hot, and applying the biuret reaction to the filtrate.

Mucin in minute quantity is a normal constituent of urine, being derived from the mucous lining of the bladder and urinary passages. Mucus, however, is insoluble in acid urine, and settles with the epithelial *débris* when the sample has been allowed to stand. In alkaline urine it may pass into solution, and in catarrhal conditions of the urinary surfaces it may exist in sufficient quantity to make a perceptible precipitate on the addition of a few drops of acid. This must not be mistaken for albumin, which under like conditions is not precipitated until coagulated by heat.

Blood in Urine.—Blood often appears in urine, and the condition is known as hæmaturia. In these cases all the ingredients of blood, chemical and microscopical, are found, a true hæmorrhage having occurred into the urinary system. Another condition is met with in which no blood-corpuscles are found, but only the blood-colouring matter: this is known as hæmoglobinuria. Both conditions of necessity make the urine albuminous and impart colour in proportion to the quantity present. The special test for blood most commonly used is the guaiacum test, which depends upon the wonderful oxygen-carrying power of the blood-pigment. This test, therefore, is applicable both to hæmaturia and hæmoglobinuria, and is applied as follows:

Fill a test-tube with urine to the depth of an inch, add two or three drops of tincture of guaiacum (not tr. guaiaci ammon. but guaiacum 1 in S.V.R. 20). The resin of the tincture will be thrown out, but this may be disregarded. Now add half an inch of ozonic ether without shaking, and in presence of blood a blue colour will appear at the bottom of the ethereal solution.

Pus, iodides, and saliva give similar reactions, but these do not affect the colour of the sample unless it be in an opposite direction. If any doubt exists, the presence of blood as a whole can easily be ascertained by finding the corpuscles in the deposit (*see* microscopical section), or the presence of blood-pigment may be verified by means of the spectroscope (*see* page 67). Some authorities state that the site of hæmorrhage may be discovered by the colour of the blood-stained urine, but too much reliance must not be placed upon such distinction.

They say brown-coloured urine indicates hæmorrhage from the kidneys, and red-coloured urine from the bladder, but such differences may arise from other causes. Bright blood at the beginning of micturition, followed by normal-coloured urine, suggests the urethra or prostate gland as the site. It is not safe to assume the absence of blood if it is not apparent to the naked eye, as blood-corpuscles and a faint trace of albumin are often observable when the blood is insufficient in quantity to appreciably alter the colour. Again, an acid reaction is not incompatible with considerable quantities of blood—indeed, hæmorrhage may amount to much before the alkaline blood overcomes a highly acid sample of urine.

Another blood-derivative which is sometimes found in urine is hæmatoporphyrin. It is generally due to overdoses of sulphonal. The urine is dark-coloured in consequence of the presence of this substance, which can be detected by spectroscopic examination after suitable treatment (*see* page 67).

Sugar in Urine

Grape Sugar, Glucose, or Diabetic Sugar appears in the urine in the disease known as *diabetes mellitus*, in which disease it is coupled with an unusual dryness of skin and a voracious appetite, the patient rapidly loses flesh, and ultimately, if he be young, is seized with diabetic coma and dies. Recovery is rare. Glucose also is frequently found in the urine of corpulent people past the meridian of life, but it is generally not attended with such pronounced symptoms, and is comparatively easily controlled by diet, or by diet and drugs. This disease, by way of distinction from the grave disease, diabetes, is known as glycosuria. Injuries to the brain sometimes induce sugar in the urine. Its appearance is a symptom only, and under which heading the disease should be classed depends upon concomitant symptoms and age. The actual cause of its presence is unknown. It is presumed that the liver is at fault, but no *post-mortem* examination, either microscopical or

macroscopical, reveals any difference from health in that organ.

The ordinary clinical tests for sugar should give a negative result, but it is possible, with the most delicate tests and care in their application, to get a sugar-reaction in healthy urine. If, however, a positive grape-sugar reaction is obtained by the ordinary clinical tests, a pathological condition is indicated.

The physical conditions of urine when sugar is being passed in large quantity have already been noted—namely, increased specific gravity, decreased colour and high degree of acidity, and peculiar odour; but in the absence of these it is not safe to conclude that no sugar is present, as sugar is often to be found in samples of less specific gravity than 1·015, and presenting no abnormal appearance.

Qualitative Tests

Before applying any of the tests for sugar it is advisable to coagulate and filter out albumin, if it is present. The older tests, such as Moore's and Trommer's, have been superseded and become obsolete; they are, however, of interest in the evolution of urine-analysis, and are quoted here from the first edition :—

Moore's Test.—Mix equal quantities of urine and liquor potassæ in a test-tube, heat to 212° F., and boil for a minute or two. If sugar be present, the urine will turn dark red, brown, or even black; whilst non-saccharine urine will only turn a brownish-red.

Trommer's Test.—To 1 fl. dr. of urine in a test-tube add 10 drops of a solution of sulphate of copper (10 gr. to the ounce), then add liquor potassæ drop by drop until the precipitate first formed is redissolved; slowly heat the solution to 212° F., when, if sugar be present, an orange-red precipitate, after a time becoming reddish-brown, is thrown down.

Fehling's Test is performed with the B.P. potassio-cupric tartrate solutions. Equal parts of the two solutions—say,

about a drachm in all—are boiled in a test-tube, *a like quantity* (not more) of the suspected urine is added, and the mixture boiled again for a few seconds. In the presence of more than 1 gr. per oz. of sugar in the specimen a precipitate of suboxide of copper will be thrown down immediately, varying in colour from light yellow to brick-red, according to the quantity of sugar present. If a quantitative test is subsequently to be applied, it is wise to add the urine a few drops at a time, and boil up after each addition. By this method a guide is given as to the necessity of dilution of the sample for the quantitative test. Fehling's test has its weak points, for it reacts with other substances besides diabetic sugar; also, if the two solutions are mixed long before use (a week does not matter), it becomes too sensitive, and deposits copper oxide simply on boiling. Some of the normal urinary ingredients reduce Fehling's solution to a slight extent—for example, uric acid and creatinine—and the administration of certain drugs causes the urine to reduce although sugar is not present. Chloral is a notable offender, and chloroform has a like effect. What has been said before about peptones and albumoses and the biuret reaction will put the reader on his guard should the test-solution turn red without depositing cuprous oxide. It will be seen from this that although one may always rely on Fehling's solution not to miss any clinical quantity of sugar, on the other hand it may mislead by giving a reaction when sugar is absent. The phosphates of the urine are thrown out by the alkali, but they produce a white cloud, not a yellow or red precipitate. If necessary they may be removed, before testing the sample, by the addition of a few drops of potash solution and filtering. But better still in case of doubt is to adopt the plan of Mr. A. H. Allen, which gets rid of almost any interfering substance. It is conducted as follows :—

From 7 to 8 c.c. ($=\frac{1}{4}$ oz.) of the sample of urine to be tested is heated to boiling in a test-tube, and without separating any precipitate of albumin which may be produced, 5 c.c. (about 80 m) of the solution of cupric sulphate used for preparing Fehling's solution is added. This produces a precipitate containing uric acid, xanthine, hypoxanthine,

phosphates, &c. To render the precipitation complete, however, it is desirable to add to the liquid, when partly cooled, from 1 to 2 c.c. (20 to 30 m) of a saturated solution of sodium acetate having a feebly acid reaction to litmus. The liquid is next filtered. To the filtrate, which will have a bluish-green colour, 5 c.c. (80 m) of the alkaline-tartrate mixture used for preparing Fehling's solution is next added, and the liquid boiled for fifteen to twenty seconds. In the presence of more than 0·25 per cent. of sugar separation of cuprous oxide occurs before the boiling-point is reached, but with smaller quantities precipitation takes place during the cooling of the solution, which becomes greenish, opaque, and suddenly deposits cuprous oxide as a fine orange-yellow precipitate.

Picric-acid Test. — This is another method which may be applied in case of Fehling's test giving a dubious reaction. Picric acid or trinitrophenol when boiled in alkaline solution with a reducing-agent is converted into picramic acid or triamidophenol, thus :

$$C_6H_2(NO_2)_3OH + 9H_2 = C_6H_2(NH_2)_3OH + 6H_2O.$$

This picramic acid in solution is of a deep reddish-brown colour, consequently in presence of glucose the yellow mixture of urine and picric acid is converted into a deep reddish brown. Unfortunately, normal urine reduces picric acid to a slight extent by reason of the creatinine which it contains ; this, however, is so slight that the resulting fluid is still transparent and only light red, while if sugar be present, a test-tube of $\frac{3}{4}$ inch diameter containing the mixture is so dark red that light is not transmitted through it.

Other coal-tar products, such as methylene blue, nitropropiol, and safranine, are also used, and deservedly obtain a great measure of favour. They possess an advantage over picric acid in that by reduction their colour is discharged instead of being intensified, and they are not affected by creatinine, chloral, or other normal and abnormal urinary ingredients.

Fermentation Test. — In days gone by the court of appeal in sugar-testing was fermentation, but this has given way to phenylhydrazine hydrochloride. The fermentation test is easily applied, and is perfectly satisfactory if the sugar

amounts to more than 2 or 3 gr. per oz., but if less than this amount is present it is not sufficiently sensitive in consequence of the solubility of the CO_2 formed. German yeast is the ferment used, and the reaction is represented as follows :—

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2.$$

To conduct the experiment, take a large test-tube, drop into it a fragment of yeast about the size of a pea, and then half fill the tube with urine. Next shake vigorously so as to break up the yeast, for fear of bubbles of air being imprisoned within it, and then fill the test-tube completely with urine (a 3-oz. vial serves for the test in the absence of a large test-tube). Now invert the tube over a porcelain dish containing some more of the urine, in such a manner as to prevent any of the urine escaping, and place the full tube, mouth downwards, in the urine in the dish, in a warm place for twelve hours. If sugar is present, fermentation proceeds, and the CO_2 will collect in the upper part of the tube.

But this test fails in just those cases where an appeal is necessary—namely, in suspected traces, when one may have misgivings about the results of Fehling's test. In such cases one must fall back upon the test next described.

Phenylhydrazine Test. — Phenylhydrazine forms with glucose a compound known as phenylglucosazone, which is almost insoluble in cold water, and separates from hot solution in well-defined crystalline form. These crystals are yellow in colour, and generally arranged in radiating clusters of needles. To perform the test, put about 10 c.c. of the suspected urine into a test-tube, and add to it about 0·4 gramme of phenylhydrazine hydrochloride and a like quantity of sodium acetate ; immerse the tube in a water-bath, and boil for half an hour ; remove the tube and set it aside to cool. In the presence of sugar the typical crystals of phenylglucosazone separate, and are easily identified by examination under the microscope. Occasionally, instead of crystals an amorphous powder is deposited, in which case it is necessary to dissolve the precipitate in hot alcohol, dilute with water, boil off the alcohol, and set

the aqueous solution aside to cool. If the first precipitate was due to sugar, the recrystallisation should result in a crop of well-formed crystals. A second crystallisation is seldom necessary. This test is extremely delicate, but it is scarcely one for routine use. Fehling's test is best for that, and the phenylhydrazine hydrochloride should be reserved for the occasional samples about which doubt exists.

Quantitative Determination

Copper-reduction processes again have the first place, but most of the tests described in the qualitative section may yield quantitative data. Some information may be gathered from the specific gravity alone, for it is safe to assume that if the specific gravity of the urine passed by any patient on one day was 1·040, and on another day 1·030, the quantity of sugar has been reduced per oz., and if the total volume in the day was the same on both occasions, that the daily elimination has been reduced. But this is unreliable except in a very broad sense and when the quantity of sugar is high. Another approximate method is by fermentation—either by measuring the quantity of CO_2 evolved or by noting the difference of specific gravity before and after fermentation. In the former manner each c.c. of CO_2 obtained = 0·002 gramme of glucose in the quantity fermented, and in the latter each degree of gravity lost equals 1 gr. of sugar per fl. oz.

Optical Method. — Diabetic sugar is dextrorotatory, therefore by introducing a column of saccharine urine into a polarimetric apparatus and measuring the rotation of the polarised ray, the strength of the solution can be readily and quickly ascertained. Polariscopes of various kinds are used for the purpose, and will be found described in books on physics. The readings are sharpest with a half-shadow instrument worked with monochromatic light obtained from a sodium flame, when the angular rotation for diabetic sugar = 52·7°. In the transition tint instruments, in which daylight or ordinary artificial light is used, the rotation of the mean ray

= 57°. Some saccharometers are graduated to read percentage of cane sugar in solutions of definite strength instead of degrees of rotation. With these it is necessary to make a table of degrees corresponding to the figures engraved upon the instrument. The polarimeter would be all that could be desired for quantitative estimations were it not for the fact that urines commonly contain other substances than sugar which rotate the plane of polarised light—in fact, lævorotation of variable degree is the normal effect of urine. Consequently, the determination cannot be worked out to refined nicety, the lævorotation being great enough sometimes to account for 0·5 per cent. of dextrorotatory sugar before right-hand rotation begins. Another drawback exists in the fact that when the sugar does not amount to more than 5 or 6 gr. per oz. it often exists in high-coloured urines which in a length of 100 cm. are too opaque for the ray to penetrate. It is then necessary to decolorise the sample with acetate of lead by adding about 3 grammes per 100 c.c., stirring well, and filtering, when the filtrate will be sufficiently clear for the test.

Volumetric Methods by Fehling's solution and modifications thereof are easy and more accurate than the foregoing. To estimate by Fehling's solution, take 10 c.c. of the mixed solutions (5 c.c. of each) in a white porcelain dish, and dilute with 40 c.c. of distilled water. Charge a 25-c.c. burette with the sample of urine, diluted from two to ten times with distilled water according to the strength of the solution as ascertained approximately by a qualitative test, or as indicated by the specific gravity. Whilst the test-solution is boiling in the porcelain dish, run in the urine solution from the burette until all blue colour disappears. To ascertain this it is necessary to withdraw the flame from time to time and let the cuprous oxide settle, then cant up the dish, when the blue may be seen against the white dish at the margin. Another and better plan is to stuff a plug of absorbent cotton into the end of a piece of glass tubing about 6 inches long and $\frac{1}{4}$ inch in diameter, and suck about $\frac{1}{4}$ inch of the fluid in the dish

through the wool. The wool, if nicely packed, will filter out the copper oxide, and if any blue colour is visible in the tube, blow it back into the dish and add another quantity of urine solution. This plan works well and prevents the bumping consequent upon allowing the copper oxide to settle. When the final reaction is reached, read off the quantity of urine solution that has been used, and that will contain 0·05 of glucose or diabetic sugar, and calculate by the following equation :—

$$x = \frac{0{\cdot}050 \times 100 \times d}{n}$$

where x = percentage of sugar, d = dilution of urine, n = number of c.c. of diluted urine used.

To obtain gr. per oz., multiply by 4·735; and to find the total for day, multiply this by the number of ounces passed.

Pavy's Method.—This method has distinct advantages over Fehling's, on account of the end-reaction being much easier to see. The principle of the test is the same—namely, the reduction of cupric oxide to cuprous oxide—but instead of the cuprous oxide being thrown out of solution as a red or yellow precipitate, it is retained in colourless solution by ammonia. Pavy's solution is ten times weaker than Fehling's, and consequently the equivalent of glucose for 10 c.c. is 0·005 instead of 0·05. Yet in preparing Pavy's test-solution it is necessary to take more than one tenth part of the copper used in Fehling's test, because for some reason the reducing action of the glucose is not so strong. A convenient way of preparing the solution is to mix 60 c.c. of each part of Fehling's solution, add 300 c.c. of liq. ammon. fort. '880' and 100 c.c. of caustic-soda solution, and make up to 1,000 c.c. with distilled water. The test cannot be conducted in an open dish in consequence of the rapid loss of ammonia, and also because Pavy's solution quickly absorbs oxygen from the air, and the reducing action of the glucose is reversed. It must be conducted in a flask provided with an exit tube as suggested by Mr. A. H. Allen in fig. 9. If the fumes are of no consequence, they may be allowed to blow into the air instead of

into the water, and the annoyance of a back suck is avoided. The long vertical tube is for the passage of coal-gas, so as to exclude the oxidising effect of air. This latter is hardly necessary in clinical work, especially after the operator has acquired skill enough to conduct the test regularly, and thus keep the flask full of ammonia-vapour. A white screen or

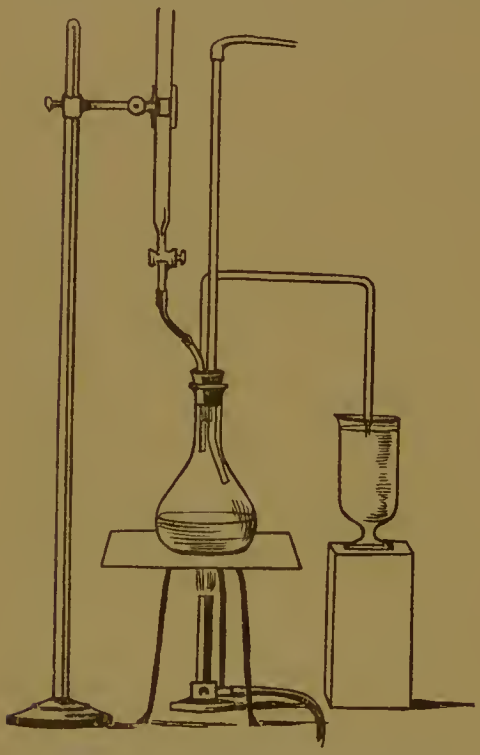

FIG. 9.—ARRANGEMENT OF APPARATUS FOR PAVY'S METHOD (*after Allen*).

FIG. 10.—APPARATUS FOR GERRARD'S METHOD.

sheet of paper behind the flask makes the end-reaction (final disappearance of the blue) more sharply visible. The experiment must not be conducted too fast; the tap of the burette should be regulated to discharge drops at the rate of about 100 per minute. The sample of urine, too, must be more dilute than with Fehling's test, in correspondence with the greater weakness of this test. Some chemists use a greater

volume of Pavy's solution, in which case of course the calculation must be made accordingly, but with 10 c.c. the equation is as follows, x being quantity of sugar :

$$x = \frac{0{\cdot}005 \times 100 \times d \text{ (times diluted)}}{n \text{ (c.c. used)}}.$$

A burette for sugar-estimations has been devised by Mr. A. W. Gerrard, which is graduated in percentage figures instead of cubic centimetres. This is a great convenience, as it not only saves a calculation, but eliminates a possible mistake in doing it. Not only is Gerrard's glycosometer a useful burette, but so also is a method of using Fehling's solution which bears his name. It will have been noted that in using Pavy's method the hot solution is in a corked flask, to screen it from the influence of the air, and the escaping steam and ammonia-gas are led into a vessel of water (*see* fig. 9). All this complicates the operation somewhat, and the ammonia-fumes are undesirable, and often, in fact, not permissible in the laboratory. Hence an alternative method, having an end-reaction like Pavy's and without these drawbacks, is very much to be desired. Such a one is Gerrard's. Instead of the cuprous oxide being precipitated as in Fehling's test, it is kept in solution as in Pavy's, but the solvent is double cyanide of copper and potassium. A 5-per-cent. solution of potassium cyanide must be prepared. The method is as follows :

Charge the burette with the urine diluted from two to ten times as in using the ordinary Fehling's test. Prepare 10 c.c. of Fehling's solution (5 c.c. of each part) and boil it in the porcelain dish, then, while boiling, add to it, by means of a pinch pipette, sufficient of the solution of potassium cyanide to discharge all the colour, taking care not to overstep the mark. Now add to the still boiling liquid another 10 c.c. of Fehling's solution and sufficient water to make the total about 50 c.c. As soon as the whole boils, run in the saccharine urine from the burette until the colour is completely discharged. The quantity of urine required will contain 0·05 of glucose, and the calculation is the same as in Fehling's test.

The whole test should be done as expeditiously as possible, for although it may be done in an open dish oxidation does take place slowly. Again, the potassium-cyanide solution is apt to turn slightly brown, and consequently the end is not

quite so sharp as in Pavy's method ; but with a little practice and a good eye the disappearance of the last trace of blue can readily be made out. It is a good plan to read the burette when you think the blue has all gone, and then add another drop or two of the diluted urine, and note the effect. The advantages of this method make it the most desirable one to adopt for clinical testing.

Acetonuria

Acetone occurs sometimes in the urine, chiefly in diabetes. If it is present in much quantity, it imparts its peculiar odour to the fluid. In order to demonstrate its presence it is sometimes necessary to distil the sample and test for the acetone in the distillate. If, however, there is much acetone, it can be detected in the sample without distillation. The test is as follows: Fill a test-tube to the depth of 1 inch with the urine, add 2 or 3 drops of strong fresh solution of sodium nitroprusside and a few drops of potash solution. A red colour is produced, which in the course of a few minutes becomes yellow. But without waiting for this change add an excess of acetic acid, and in presence of acetone a rich crimson red with a tinge of violet is produced.

To detect acetone in the distillate the iodoform test is the best. Take 100 to 200 c.c. of the sample, acidulate strongly with acetic acid, and gently distil about 10 c.c. To the distillate apply the iodoform test as follows: Add about 10 to 15 drops of potash solution and warm it up to about 50° C.; now add, drop by drop, a solution of iodine in aqueous potassium iodide until a permanent colour just appears ; next add a drop or two more of the potash solution until the colour is again discharged. If acetone is present, a precipitate of iodoform will appear, according to the following equation :

$$CH_3 \cdot CO \cdot CH_3 + 4KOH + 3I_2 = 3KI + CH_3 \cdot CO \cdot OK + CHI_3 + 3H_2O.$$

The significance of acetone in the urine coupled with sugar is grave, particularly when aceto-acetic acid (which *see*) is also

present. Prompt measures may avert a fatal termination, but it is generally looked upon as a sign of approaching diabetic coma. Acetonuria, however, is not found in diabetes only, but in some febrile diseases and in excessive alcoholic indulgence. It is not indicative of such a grave condition in these cases. It is necessary to remember that acetone has a reducing action upon Fehling's solution, and to guard against mistake on that score boil the urine (with which the Fehling's test is to be made) for a minute or two to blow off the acetone.

Aceto-acetic Acid

Much importance is attached to a reaction with ferric chloride, which is observed sometimes in diabetic urine. It is said to be due to the presence of aceto-acetic acid. This is a body closely allied to acetone, as may be seen from the formula, $CH_3COCH_2CO{\cdot}OH$. To test for aceto-acetic acid fill a test-tube to the depth of 1 inch with urine, add a few drops of solution of iron perchloride (B.P. test solution); at first a bulky precipitate of phosphate of iron will be thrown down, but on further addition of the iron solution a dark-red colour will be struck if acetone is present. Some recommend filtering off the iron phosphate, but it is not essential. It is necessary, however, to remember that some drugs cause a similar reaction, notably salicylates, phenol, and antipyrin. To discriminate between the deep red produced by these and aceto-acetic acid it is necessary to boil the contents of the test-tube, when the colour produced by the former substances will persist, while that produced by the latter will fade away. This is due to the instability of aceto-acetic acid, which is destroyed by boiling. A sample, therefore, to be tested for this substance should be fresh, and not boiled previous to the test.

Bile

Bile may exist in the urine either as bile pigment or bile acids, or both. Its presence makes the urine greenish brown, the depth of colour depending upon the amount of bile

present, and the urine also becomes rather more viscid and frothy, the froth being tinged yellow and very persistent. The yellow colouring-matter is also taken up very readily by white clothing, and thus stains the linen of patients suffering from bile in their urine. This tinctorial property is made use of in one of the tests for bile pigment.

Gmelin's Test is very commonly used. Into a test-tube pour to the depth of half an inch nitric acid containing nitrous fumes (a splinter of wood—a match—in some pure nitric acid soon provides this), and, holding at an angle, pour gently a little of the suspected urine in such a way that it shall run down the side of the tube and over the surface of the acid without mixing. At the line of contact a zone of red appears in every sample of urine, but if bile pigment be present there will be a series of zones, green being uppermost, with violet, red, and yellow in succession below. The play of colours is not permanent, but soon fades away, and the essential colour is the green : other substances may produce the rest. Sometimes the play of colours is better observed by filtering a portion of the sample and applying the fuming nitric acid by means of a glass rod to the moist filtering-paper. The paper will have absorbed the bile pigment, and the zones of colour will surround the spot of nitric acid. Bile acids may be sought for by Pettenkofer's test, or better by Oliver's test.

Pettenkofer's Test for Bile Acids.—Mix urine with half its bulk of strong sulphuric acid in a test-tube (rise of temperature being prevented by immersing the tube in water). A small quantity of white sugar is then added and well mixed with the acid and urine ; more sulphuric acid is now added, and as the temperature rises a reddish or violet coloration is produced, the liberated cholalic acid due to the decomposition of the taurocholate and glycocholate of soda furnishing the colour. The presence of albumin or volatile oils interferes with this test.

Oliver's Test for Bile.—Take of peptone 2 grammes, acid. salicyl. 0·25 gramme, acid. acetic. 2 c.c., aq. dest. up to 200 c.c. Filter till clear. Dilute the sample of urine down

to at least specific gravity 1·008 by the addition of distilled water. If it is specific gravity 1·016 it will want an equal quantity, if specific gravity 1·024 it will need double, and so on. The urine must be perfectly clear. One c.c. of the diluted urine is now added to 3 c.c. of the peptone solution, and in presence of bile salts an opalescence will be formed whose density is proportional to the amount of bile present.

The presence of bile pigments and bile salts in the urine is indicative of disease of the liver, from simple jaundice to the gravest disorders.

Other Abnormal Constituents

Indican, or potassium indoxyl sulphate, is a salt which appears in the urine and imparts to it a dark-brown colour. It is easily decomposed by oxidising agents into indigo-blue and acid potassium sulphate, and this is the method adopted for its detection. Pour urine into a test-tube to the depth of an inch, add a like quantity of hydrochloric acid, and, drop by drop, liquor calcis chlorinatæ. In presence of indican a blue colour appears. Add a little chloroform, and shake, when the indoxyl will dissolve in and separate with the chloroform. It is important to add the liquor calcis chlorinatæ very carefully, as an excess bleaches the indoxyl and spoils the test. Some prefer to use nitric acid instead of the chlorinated lime because its oxidising effect is less energetic, and consequently the risk of overdoing it is lessened.

Indican is found in a great variety of conditions more or less important. For example, it is formed when albuminous foodstuff putrifies in the bowels, the soluble potassium indoxyl sulphate being absorbed and eliminated by the kidneys. Such a condition may arise from nothing more serious than constipation. On the other hand, diseases of the bowels, such as tubercular disease or typhoid fever, may be the cause, or large abscess-formation in any part of the system may result in the formation of indican.

MICROSCOPICAL EXAMINATION

For urinary work a microscope with a $\frac{1}{4}$-inch objective is perhaps the best power; no sub-stage condenser is necessary, but a small stop in the diaphragm, and not too bright a light. To prepare the specimen for examination it is a good plan to pour the sample into a conical glass of 6 or 8 oz. capacity, and leave it to stand overnight, during which time the solid matter in suspension settles to the bottom. The appearance and quantity of the sediment should be carefully observed and noted—*e.g.*, 'slight flocculent deposit,' 'bulky brickdust deposit,' or whatever it may be. Much information may often be gained, after a little practice, by the naked-eye appearance of the deposit; amorphous urates and uric acid being easily recognised in acid specimens, and phosphates, pus, and blood in alkaline samples. The sediment may often be subjected to physical and chemical tests as well as microscopical examination. Urates dissolve by heat, phosphates by the addition of an acid; pus is more tenacious than either, and becomes jelly-like upon the addition of caustic alkali; and blood looks like coffee-grounds. To obtain a sample of the sediment for the microscope, take a pipette (a foot of thin glass tubing drawn out at one end to an opening that will just admit a pin does admirably), and, holding the forefinger over the end, plunge the point through the specimen to the bottom; release the finger for a moment while some of the sediment runs into the pipette, apply the finger again, and withdraw the pipette with its contents. Wipe the outside of

Fig. 11.
Deposit of Triple Phosphate.

the tube, then allow a drop of the sediment to run out on to a clean microscope-slip, apply a cover-glass, and sop up excess of fluid with filter-paper. For description the deposits are conveniently divided into unorganised and organised. The unorganised may be further divided into two classes—those found in acid specimens and those found in alkaline.

Unorganised Deposits from Acid Urines

Amorphous Urates.—These, as their name implies, are salts of uric acid, probably quadriurate of sodium and potassium, of no definite crystalline form. They appear under the microscope as small granules more or less cubic. Warming the slide by passing it two or three times through a Bunsen flame rapidly dissolves them, as also happens if a drop of alkali be run under the cover-glass. To do this place a drop of potash solution on one side of the cover-glass, and draw it under by placing a fragment of filter-paper against the opposite side. By this means the effect of the reagent may be watched while the object is still upon the stage and under observation through the microscope. Amorphous urates are a very common sediment; they frequently take up colouring-matter, and consequently look pink, hence the term 'brick-dust deposit.' This pink colour is not, however, a constant feature, as pale urines frequently deposit an almost white urate. Their presence often alarms a patient, but they cannot alone and of necessity be looked upon as of much clinical importance. Their precipitation does not always mean excess, as low atmospheric temperature, concentration, and acidity are all factors that influence their passing out of solution. A figure of urea crystals is given on page 15.

FIG. 12.
ACID SODIUM URATE.

Acid Sodium Urate is sometimes found in deposits. Microscopically it consists of spheres of small size, with spicules sticking radially from their surface (*see* fig. 12).

Uric Acid.—To the naked eye uric acid is often visible, and presents the appearance of grains of Cayenne pepper. The colour is not inherent to uric acid, but, like the urates, it is absorbed from the urinary pigments. Its presence, again, does not of necessity imply excess. The microscopic appearance of uric acid varies very much, as it is capable of crystallising in a great variety of forms. The commonest is a rhomboid, sometimes with the angles rounded off, and these are apt to adhere in clusters, edge to edge, by their ends, forming a rosette. They vary in thickness, so that edgeways they may appear as plates or as barrels ; sometimes they are arranged in

FIG. 13.—URIC ACID.

rosettes edgeways up, or even adhering by their middles as a kind of sheaf. Another common form is a dumb-bell (*see* fig. 13). It is seldom safe to base on the deposit an opinion as to quantity of uric acid : a quantitative determination is the only reliable guide. If doubt exists about microscopic crystals, whether they be uric acid or not, run a little potash solution under the cover-glass, when uric-acid crystals will dissolve. A uric-acid sediment may be verified by decanting the urine, washing the sediment with acidulated water, and again with alcohol, transferring the washed sediment to a porcelain dish, and applying the murexid test.

Hippuric Acid.—Crystals of hippuric acid are occasionally met with in the microscopical examination of urinary sediments, but they are very rare. They are generally due to the administration of benzoates or free indulgence in certain fruits. They, like uric acid, are soluble in alkalies and insoluble in acids; but they are of different crystalline form—four-sided prisms. This acid does not give the murexid test.

FIG. 14.
HIPPURIC ACID.

Calcium Oxalate.—Crystals of oxalate of lime are extremely common and generally very easy to detect. They are as a rule in the form of small octahedra, colourless and brilliantly refractile. They suggest by their shape and markings the back of a square envelope. Oxalates also sometimes assume a dumb-bell form of small size, and occasionally they look like a red blood-corpuscle. The disc-shaped crystals are generally accompanied by the ordinary envelope pattern, and they are distinguishable from blood-discs by their varied size and shape. The presence of calcium-oxalate crystals cannot be looked upon as of much pathological importance, as they are frequently found in health, particularly after eating tomatoes and other foods rich in oxalates. They should, however, be noted, as some authorities say they are increased in gastric disturbances. Calcium-oxalate crystals are insoluble in acetic acid, but rapidly dissolve in mineral acids.

FIG. 15.
CALCIUM OXALATE.

Cystin.—Cystinuria is a rare condition. When it does occur, the cystin, which is a sulphur-containing nitrogenous body, is usually abundant, and passes out of solution as a bulky yellowish deposit. It is easily decomposed and gives rise to a sulphuretted-hydrogen odour. Under the microscope cystin has a characteristic form. The six-sided

tables are soluble in mineral acids, caustic alkalies, and ammonia. If some of the deposit be dissolved in potash solution, and a few drops of sodium nitro-prusside solution be added, a violet colour is produced. The appearance of cystin in the urine is but little understood : it often runs in families. It sometimes enters into the composition of urinary calculi.

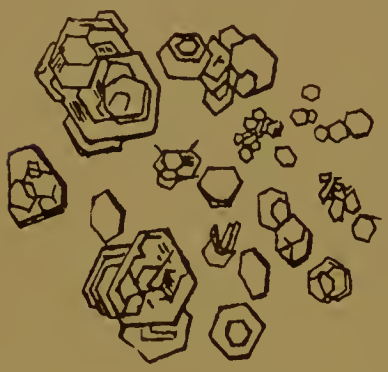

FIG. 16.—CYSTIN.

Leucin and Tyrosin.—These are very rare deposits, and are usually found associated in acute yellow atrophy of the liver, and frequently in small-pox, typhoid fever, and acute tuberculosis. Tyrosin crystallises in bundles of shining delicate needles, the bundles being sometimes arranged in a stellate form. Leucin appears in gland-like masses, circular oil-discs, or dark globular crystals. For methods of isolating these bodies and the chemical tests the reader is referred to larger works.

Unorganised Deposits from Alkaline Urines

Triple Phosphates.—The commonest deposit of this class is the ammonio-magnesium phosphate, often called triple phosphate. It may even occur in urine which is feebly acid to litmus. Triple-phosphate crystals are often of large size (*see* fig. 11), and are said to resemble coffin-lids—an uncomfortably suggestive idea, and not very accurate, as the sides are parallel ; another form of triple-phosphate crystals is the snowflake form. They are immediately soluble in acetic acid.

Basic Calcium Phosphates, also called stellar phosphates from their common arrangement, are wedge-shaped crystals, which have a great tendency to unite by their apices, thus

forming a star with the bases outwards. These, like the triple-phosphate crystals, immediately dissolve in acetic acid.

Ammonium-urate crystals are small, dark, globular bodies beset with spicules, soluble in acetic and hydrochloric acids with ultimate formation of uric-acid crystals.

Calcium Carbonate is deposited from alkaline urines as amorphous particles soluble in acetic acid with evolution of carbonic-acid gas, which forms bubbles under the cover-glass.

Organised Urinary Deposits

These are quite a different class of substances : they consist either of cellular elements or the products of living cells. In ordinary healthy urine, after standing for some time, a light cloud appears and generally settles to the bottom. This consists of mucus derived from the mucous membrane of the urethra and bladder, together with the *débris* of epithelium which is constantly being shed in small quantity from the whole surface of the urinary tract. These epithelial cells vary in size and shape in the various parts of the urinary system, and a knowledge of their appearance is of great importance in the microscopical examination.

In morbid urines epithelium may appear in greatly increased quantity, and the kinds and quantity of epithelial cells should be carefully noted—*e.g.*, whether much or little kidney epithelium. Pus-cells and blood-corpuscles must be detected with precision, and casts of the uriniferous tubules of the kidney must be sought for with the greatest care. Spermatozoa must be recognised, possibly fragments of malignant tumours may be present, and micro-organisms and parasites of larger growth must be remembered. Besides these, the observer should be on his guard for foreign bodies, which may be legion, and which are introduced by carelessness, wilfulness, or inability of some people to be precise and clean. Great attention should be paid to

this section of urinary work, as it is, perhaps, the most important of all in diagnostic value, and demands the utmost tact, skill, and patience in its prosecution.

Epithelium

The varieties that require recognition are: (1) kidney epithelium—(*a*) from the tubules, (*b*) from the pelvis of the

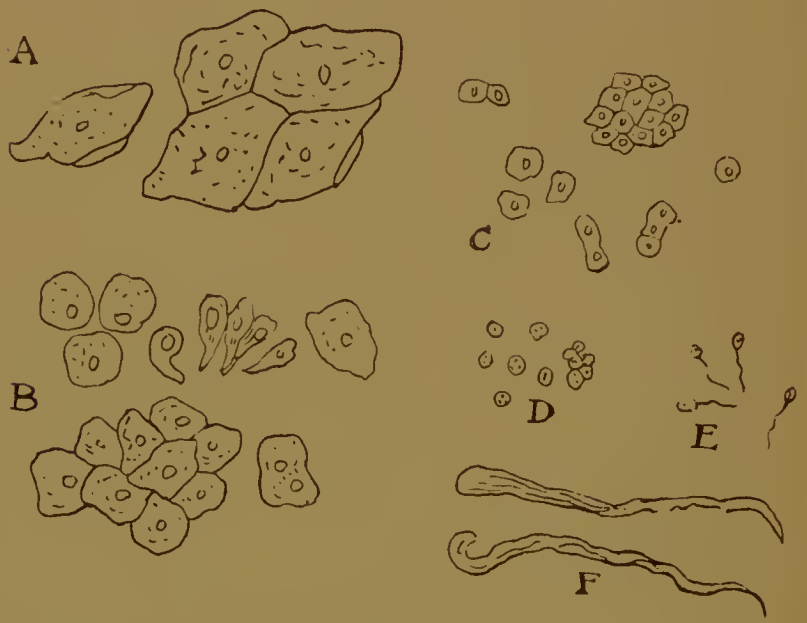

FIG. 17.

A, Vaginal Epithelium. B, Bladder Epithelium—Spherical, Spindle-shaped, and Polygonal. C, Kidney Epithelium. D, Pus-cells. E, Spermatozoa. F, Cylindroids.

kidney; (2) epithelium from the urinary tract, ureter, bladder, and urethra; (3) epithelium from the vagina and prepuce. These cells may appear in small number in healthy urine, but if there is any inflammatory process going on in any of the areas named, desquamation is greatly increased at that part, and the locality may be ascertained by its kind of epithelium being much increased in the urinary deposit.

Kidney Epithelium from the uriniferous tubules consists of round cells, slightly larger than a pus-cell, with a single nucleus of large size and, possibly, prominent nucleoli. They are generally isolated (if not associated with casts), and frequently undergoing fatty degeneration, indicated by the existence of bright dots of fat in the protoplasm of the cell. This kind of epithelium seldom appears in large quantity, and is of great pathological importance. Its presence is a sign of renal disease, and when fatty degeneration is well marked suggests a like change in the general substance of the kidneys.

Renal Epithelium from the pelvis of the kidney is similar to the above, but the outline of the cells is often polygonal instead of round, and the cells are adherent to one another in clusters rather than isolated. Its presence indicates inflammation—pyelitis—and the kind of inflammation may be suggested by the accompaniment of pus-cells, blood-corpuscles, or crystals. In the former case, other clinical symptoms may point to tubercular disease, and special staining process may reveal the tubercle bacillus. Epithelium of this class, with hæmorrhage and crystals, or particularly lumps of crystals, may establish the presence of stone in the pelvis of the kidney.

Bladder Epithelium is similar to epithelium from the ureters and upper portion of the urethra: the site of inflammatory process depends largely upon quantity. The superficial area of the bladder, being much greater than the other parts, yields a correspondingly greater quantity of epithelium—moreover the ureters are less prone to inflammation, and diseases of the urethra can generally be detected by other means. There is no constant form for bladder epithelium: its shape and size vary. It is generally spoken of as consisting of three layers. The upper or surface layer is represented by cells of large size, polygonal, round, or broadly oval in shape, with one or more large nuclei, and often with granular protoplasm. They are frequently isolated, but as many as ten or a dozen may be found adherent by their

margins. The middle layer consists of more irregular narrow cells, sometimes oval at one end, and tailing off in one or more processes at the other. The deeper layer is more cylindrical and smaller. The superficial layer must not be mistaken for the next class.

Vaginal Epithelium is found very frequently in the urine of women, and often in large quantity. In smaller quantity, cells of like appearance may be derived from the meatus and prepuce of the male. These epithelial cells are larger than bladder epithelium, are generally mononuclear and united in large flakes by their margins. The free margins are very prone to curl.

Pus is a common pathological ingredient in urine. The pus-cells settle down with the deposit, and are found in the microscopical examination. They may be so numerous as to form a deep layer of sediment, as when an abscess opens into the urinary tract or in acute cystitis, or they may be so rare as to yield only a few in a microscopic field. But no matter how few, they should be noted. The presence of pus implies the presence of albumin, as pus is albuminous. Pus indicates an inflammatory process, and the seat of this process must be sought for by the evidence afforded by other elements. Pus and tube casts point to renal disease, pus and epithelium from the pelvis of the kidney to pyelitis, pus and bladder epithelium to cystitis, fœtid pus in large quantity to abscess. Pus also is found in urethritis and leucorrhœa, and in small quantity commonly in gouty people. A sediment containing much pus is not readily shaken up, is whitish unless blood be also present, is apt to turn ropy, and on the addition of potash solution may become quite gelatinous. Pus-corpuscles under the microscope are rather larger than blood-corpuscles, are colourless and spherical, and contain two or three nuclei. The nuclei often require the addition of a little acetic acid to bring them out.

Blood, like pus, may be present in quantities varying from almost pure blood down to minute traces evident only by the closest observation. In large quantity it makes

a deposit resembling coffee-grounds, while in small quantity it may only amount to a few corpuscles in a microscopic field, and be far too little for naked-eye detection. One must never assume that blood is absent because it is not evident at sight, nor even because the chemical tests fail. The detection of blood by the microscope depends upon the presence of blood-corpuscles. The ordinary microscopic appearance of red blood-corpuscles (fig. 18) needs no description—if a beginner in urine-analysis is unfamiliar with it, he should not remain so. In urine the corpuscles are seldom found in rouleaux, and the difference in the specific gravity and reaction of the urine and the blood-plasma may cause some alteration in the shape of the blood-corpuscles—they may be swollen or crenated ; they are, however, generally easily recognised.

FIG. 18.
BLOOD-CORPUSCLES.

But not so easy is it to trace them to their source. As in the case of pus, it is necessary to search for sidelights to illuminate the question. If tube casts are found, the hæmorrhage is probably from the kidneys ; if there is pus also (abundance of leucocytes) and epithelium from the pelvis of the kidney, it is probably from that region ; if accompanied with much bladder epithelium and mucus, the inference is inflammatory condition of the bladder. Hæmorrhage due to enlarged prostate gland and injuries to the urethra is often detected at the time of micturition, as it is not intimately mixed with the urine before leaving the body, but occurs as bright blood either at the beginning or end of the act. Hæmorrhage is sometimes due to an abnormal growth. Malignant tumours may form in the urinary tract, notably in the bladder, and, breaking down, bleed into the urine. Such growth may occasionally be detected or inferred through portions of the growth being carried away with the urine. Papillomatous growth (villous bladder), benign in itself yet often fatal from the hæmorrhage it

causes, may sometimes be detected through portions becoming detached and appearing in the urinary deposit.

Casts of the uriniferous tubules, as their name implies, are moulds of some material, probably a derivative of albumin, formed in the tubules of the kidneys, which, becoming detached, are carried away in the urine and are found in the urinary sediment. They are bodies of the utmost importance in diagnosis, and every effort should be made for their detection. Various writers have stated that they are to be found in non-albuminous urines, but this is not the experience of the writer, who believes that true tube-casts are invariably the outcome of albumin.

FIG. 19.—TYPES OF RENAL TUBE CASTS (much magnified). A, Hyaline. B, Granular. C, Epithelial. D, Hyaline with Pus. E, Hyaline with Epithelium. F, Blood. G, Waxy.

Casts are found of very various kinds, and attempts have been made to classify them. The classes are useful for description, but as casts are found which combine the features of two classes or more, the distinctions are more arbitrary than scientific. The simplest division is into hyaline casts, waxy casts, granular casts, and cellular casts.

Hyaline Casts are homogeneous, transparent moulds of the uriniferous tubules of varying diameter and length. They are often so transparent that they are not easily seen with a bright illumination, hence the desirability of conducting the microscopical examination without bright light and with a small stop in the diaphragm. In samples containing only a faint trace of albumin, half an hour or more may be spent in the search

before hyaline casts can be detected. They do not of necessity mean disease of the kidneys.

Waxy Casts are more rare. They are more easily seen than hyaline casts, are generally of larger diameter, they appear denser and flatter, and are often fissured transversely. They are not well understood, but it is clear that they are not invariably associated with amyloid changes in the kidney.

Granular Casts appear coarsely or finely granular; they are seldom found of any great length, but frequently in mere fragments. They must not be confounded with crystalline casts or with degenerated epithelial scales, which sometimes simulate them closely. They are said to be a reliable indication of nephritis.

Cellular Casts may be casts beset with pus-cells or kidney epithelium, or may be composed of blood-corpuscles. The cells may compose the whole cast, or they may be only dotted about here and there; or a cast may be hyaline for half its length and cellular the other half. Epithelial casts prove disintegration of the kidney tubules, and imply acute nephritis. Blood-casts, with casts containing pus, indicate acute renal inflammation, but the blood-casts alone may only imply renal hæmorrhage.

Besides true casts, spurious casts are found in urine which are often so close in their resemblance as to make distinction difficult. In forming an opinion it is necessary to take a broad view of the case; if undoubted casts are found, the observer may assume that the doubtful ones are true casts also, but if the specimen has lots of doubtful ones he should hesitate about reporting renal casts. These spurious casts are called cylindroids; they are commonly long and doubled, and tail off at their extremities, or at least at one extremity. They probably consist of mucus (*see* fig. 17, F).

Mucus itself is often present in considerable quantity, particularly in catarrh of the bladder. It is seen under the microscope in irregular, wavy, transparent shreds.

Spermatozoa may occur in the urine: they are easily recognised by their pear-shaped head and long, whip-like

tail (fig. 17, E). Some tact should be used in reporting their presence, as they may exist under perfectly normal conditions. Their detection may, however, be a link in the evidence of a vicious habit.

Micro-organisms.—Various micro-organisms of non-pathogenic nature are to be found in any urine that has been kept for some time unless strict bacteriological methods of collection have been observed. Notable among them are the *Micrococcus ureæ*, already mentioned, and a variety of bacteria commonly described as vibrios. Yeast fungus often finds its way into and grows rapidly in saccharine urine. Of the pathogenic fungi, those most commonly to be sought for are the tubercle bacillus and the gonococcus. The latter is always associated with pus, and the former generally—in fact, it is almost a waste of time to search for it unless pus be present. Typhoid-fever patients pass typhoid bacilli in their urine, which consequently is highly infectious and must be handled with due care. Various other diseases yield their characteristic bacteria, for detailed information and methods of detection of which the reader is referred to books on bacteriology. The presence of tubercle bacilli and gonococci, however, affords such valuable information to the physician that details of the processes for finding them are included in this volume.

To Detect Tubercle Bacilli.—Add to the suspected urine sufficient liquefied carbolic acid to make the solution 1 in 20, and shake vigorously. Pour the carbolised urine into a conical glass and leave it for twenty-four hours for the sediment to form. Remove a little of the sediment by means of a pipette, and place a drop of it upon a scrupulously clean microscope cover-glass of the thinnest kind. Evaporate the water, and if the residue is not very thick, evaporate to dryness by holding the cover-glass about a foot above a Bunsen flame. If the residue is thick, squeeze it out into a thin film by placing a similar cover-glass over it and sliding the two apart with some pressure. As soon as the film is dry, pass it three times through the Bunsen flame to fix it

to the glass. Wash the film for two minutes in equal parts of alcohol and ether. Dry again, and again pass through the flame. The specimen is now ready for staining. A great many methods of staining are in vogue, all depending on the remarkable avidity of the tubercle bacillus for coal-tar colours, which it retains even in spite of the action of strong mineral acids—a treatment which almost anything else will not stand. The stain most commonly used is carbol fuchsin—this stains the tubercle bacilli red, and by way of contrast it is customary to stain the rest of the specimen blue with Höffler's methylene blue. The solutions are prepared as follows :—

(1)

Conc. alcoholic solution of fuchsin . . .	10
5-per-cent. aqueous sol. of carbolic acid to . .	100

(2)

Pure sulphuric acid	1
Distilled water to	4

(3)

Conc. alcoholic solution of methylene blue . .	3
Aqueous potash (1 in 10,000)	13

Take the cover-glass in a pair of forceps, film upwards, and cover it with carbol fuchsin by means of a glass rod or pipette. Warm it over the flame until steam rises, keeping the whole surface wet the while. Wash away excess of stain in water. Decolorise in the dilute acid by dipping the cover into the acid until all the colour appears to be discharged. Next, plunge the cover-glass in 60-per-cent. alcohol and wash it in the spirit until the red colour (which at first returns) ceases to wash off. Wash again in water, dry, and again pass through the flame. Now stain with the methylene blue for a few seconds, cold. Wash in water as long as any blue washes off, dry, pass through flame, and mount on a slide with xylol balsam. With a good sub-stage condenser, flat mirror, and wide diaphragm, the tubercle bacillus can be found with as low a power as $\frac{1}{4}$ inch by an expert, but a $\frac{1}{12}$-inch oil-immersion lens is almost essential.

To Detect Gonococci.—Take some of the pus and prepare thin films by squeezing out a little between two clean

cover-glasses. Dry and fix as described above, and stain with carbol fuchsin. Wash thoroughly in water, dry and fix, and mount in xylol balsam. The gonococci will appear as minute red dots in pairs, either free or within the pus-corpuscles or the epithelial cells. A good light is essential, a flat mirror, wide diaphragm, sub-stage condenser, and $\frac{1}{12}$-inch oil-immersion lens.

Parasites of higher organisation, or their eggs, are commonly found in the urine in tropical countries, and occasionally patients suffering from them arrive in England. The commonest are *Distoma hæmatobium* and *Filaria sanguinis-hominis*. Both cause hæmaturia. Very rarely echinococcus cysts burst into the urinary tract, and hooklets and fragments of cyst-wall may be found in the urine.

Foreign Substances.—In microscopical examination of urinary sediments one must always be on guard against accidental and intentional admixture of foreign substances. The commonest are hairs, cotton and wool fibres, starch grains, and sputum. The writer was once very much bothered by a specimen that turned out to contain lycopodium, which had been used as dusting-powder, and another time by one mixed with a tooth-wash containing myrrh. Oil-globules from catheters are common. Occasionally fœcal matter finds its way into the urine by an abnormal communication between the bowel and the urinary passages, but more often it is due to accidental admixture.

OPTICAL EXAMINATION

With the Spectroscope

It is not the province of this book to describe the optical principle of the spectroscope, for which the reader is referred to works on physics or special books on spectrum-analysis. There are three kinds of instruments to select from—(1) direct-

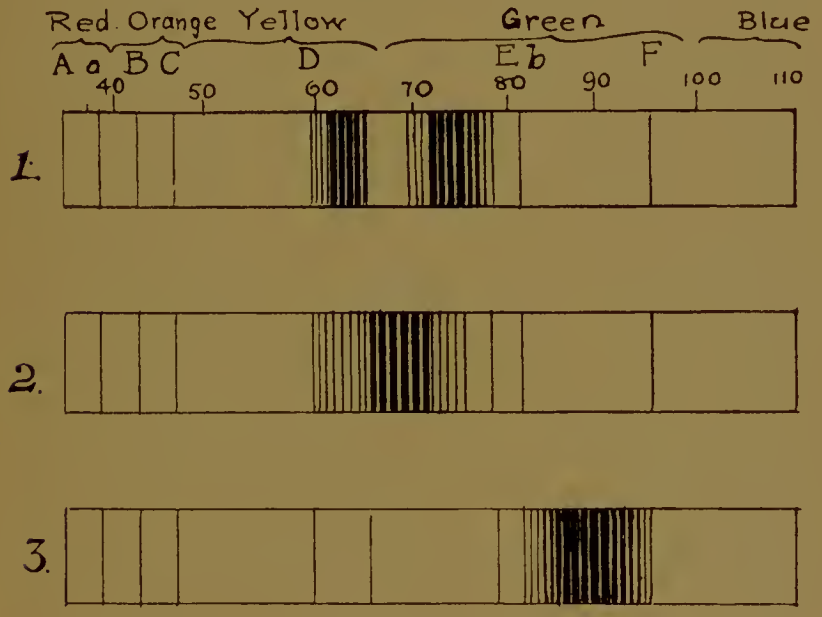

Fig. 20.

(1) Absorption Spectrum of Oxyhæmoglobin.
(2) Absorption Spectrum of Reduced Hæmoglobin.
(3) Absorption Spectrum of Urobilin in Acid Solution.

vision spectroscopes, (2) table spectroscopes, and (3) micro-spectroscopes. For urine work the cheapest is sufficient—that is, the direct-vision instrument. The term 'cheapest' means by comparison with the others, but it is wise to obtain a good

one of its kind. The urine may be examined in a test-tube, but preferably in a flat bottle—say, a ½-oz. flat white, or in a parallel-sided bottle sold for the purpose. The spectroscope should first be directed against the sky and the slit adjusted and the eye-piece focussed until Fraunhofer's lines are visible. The test-tube or flat bottle, filled with the sample to be examined, should then be placed immediately in front of the slit, and the absorption-bands observed.

Blood in urine generally yields the oxyhæmoglobin spectrum (1, fig. 20). It requires a little practice to recognise the lines in very dilute solutions, but when the observer has trained his eye the blood-bands can be picked out with ease where a novice would fail to detect them. The figure explains the position better than words, but unfortunately the reader must supply the colours mentally. The detection of these bands is almost absolute proof of the presence of blood, but, if necessary, it may be verified by the addition of a few drops of ammonium sulphide, when the two bands of oxyhæmoglobin will be replaced by the single one of reduced hæmoglobin. By exposure reduced hæmoglobin changes back to oxyhæmoglobin. Stale specimens of hæmorrhagic urine sometimes yield the spectrum of methæmoglobin, which has four bands—two in the position of the oxyhæmoglobin, and two additional ones on either side, the new one in the yellow being the most pronounced of all.

Urobilin.—Chemical methods for detecting bile-pigment have already been given. The spectroscope may also be used for the purpose. If urobilin be present in large quantity, it may be observed in the urine without treatment. If the solution is acid, there is a broad band (*see* 3 in fig. 20) between *b* and F; but if alkaline, the band moves more to the right until its centre falls at F. When, however, the quantity is small, Von Jaksch recommends the following method: Fifty c.c. of the urine is shaken up with amylic alcohol in a separating-funnel, and the urine is allowed to run off after some hours. The amylic-alcohol solution, which is more or less dark in colour, is now treated with concentrated ammoniacal alcoholic

solution of zinc chloride. If urobilin be present, the fluid assumes a beautiful fluorescence, and with the spectroscope exhibits the absorption-band of alkaline urobilin.

Hæmatoporphyrin.—This substance has received a great deal of attention lately owing to its appearance in the urine of some people after the administration of the synthetic hypnotics. Sulphonal habit may sometimes be detected in this way. Hæmatoporphyrin is a product of hæmatin free from iron. It is said to exist in the urine in minute quantity, even in health, and in increased quantities in various febrile diseases. It has frequently been observed in large quantity after excessive doses of sulphonal, trional, and tetronal, and its presence is regarded as a grave symptom. Hæmatoporphyrin darkens the urine in proportion to its quantity, and may even make it almost black. A pronounced sample, when diluted and acidified strongly with hydrochloric acid, exhibits with the spectroscope four absorption-bands (*see* 3 in fig. 20)—one faint one between C and D, a dark one at D, another faint one between D and E nearer to E, and another dark one between *b* and F. Sometimes the faint ones are not visible, but the presence of the dark bands alone is conclusive. When the quantity is insufficient for direct examination the hæmatoporphyrin may be shaken out with amylic alcohol and precipitated with ammoniacal alcoholic solution of zinc chloride in the manner described under urobilin. The precipitate should be treated with dilute hydrochloric acid, which will dissolve out the hæmatoporphyrin, and this solution will yield the characteristic spectrum.

With the Polarimeter

There is a class of crystals which do not belong to the regular system, but which have the power of double refraction; that is, when a ray of light falls upon any of them it is divided into two, one taking the ordinary course of a refracted ray, the other going in a different direction. Light so altered is said to be polarised, and the two rays are distinguished as the ordinary

and the extraordinary ray. There are many ways in which light may be polarised, but one of the most efficient is by a Nicol's prism, which is made by splitting a rhomb of Iceland spar along a diagonal plane and cementing the two parts together by Canada balsam, when one piece will produce polarisation (the polariser) and the other show it (the analyser), and every piece that serves for one of these purposes will also serve for the other. When a ray of light falls upon the first portion of the prism it is polarised—one ray, the ordinary ray, being totally reflected on meeting the Canada balsam and passing out of the prism on the other side; while the extraordinary ray, passing through the balsam and the second portion of the prism, finally emerges at the opposite end to that at which it entered.

Examined by another Nicol's prism, it will be seen that when the two prisms are placed with the chief sections parallel to each other, the ray will pass freely through; but if the analyser be turned round so that it lies at right-angles with the polariser, the polarised ray will be entirely reflected by the balsam, therefore the light will not reach the observer's eye. Certain bodies, however, such as quartz, have the power, when placed between the crossed prisms, of giving a colour instead of darkness, due to the twisting of the polarised ray from its original plane, such substances producing what is known as circular polarisation, which may be 'right-handed' (dextro-gyration) or 'left-handed' (lævo-gyration), according as it is necessary to turn the prism to the right or to the left from its proper position to produce again a complete passage of the colourless polarised ray. The rotation by all substances possessing the power of circular polarisation is fixed, and increases or diminishes according to the amount of the body present in solution.

There are two varieties of quartz, known as right-handed and left-handed. One rotates the plane of polarisation to the right, the other to the left. When a thin layer of one of these is placed between the two prisms, the ray of polarised light is rotated, and instead of being colourless is coloured, showing all the colours of the spectrum as the analyser is turned, until

it once more appears colourless ; and the extent to which the analyser has to be turned (registered by a pointer on the degrees of the circle) is the index of rotary polarisation possessed by the quartz either in a right- or left-handed direction. If the movement of the analyser be continued, colour will again show itself ; but it will be the colour complementary to that first produced. Thus, if in the beginning the colour was red between the uncrossed prisms, and by rotation at an angle of 45° there was no colour, after that the complementary colour would be green, the colour being most intense at an angle of 90°—the prisms being crossed. The polariscope used for the analysis of sugar, and spoken of as a saccharometer or polarimeter, has the following parts :

1. A Nicol's prism as a polariser.
2. A plate of quartz generally divided down the centre, one side being right- and the other left-handed.
3. A tube to contain the solution to be analysed.
4. A Nicol's prism for rotation, and having a pointer which indicates degrees of the circle on a scale in front.
5. A telescope to focus the line between the two sides of the quartz.

When the pointer is placed at zero, the tube filled with water, and the line focussed, no colour is seen on either side ; but if a solution of sugar be introduced, then a colour is produced on the side of the line corresponding to the nature of the sugar, and then the distance through which the pointer has to be moved round the graduated circle to get both sides of the quartz colourless is the degree of rotary polarisation,[1] the angle through which the prism has been turned is read off from the index and scale attached, and the amount of sugar calculated from this. *See* pages 43 and 44 for further particulars as to the use of the instrument.

[1] Working with the 100-mm. tube each degree of rotation is equal to 1 per cent. of sugar. With the 200-mm. tube the degrees of rotation divided by 2 give the percentage.

MISCELLANEOUS MATTERS

Report on Sample

It is well to have a printed form for this purpose, with the ordinary clinical data filled in and space for notes upon the sample. It should not be too bulky, and consequently it is wise not to burden the sheet with lots of matter seldom required, but to leave a few blank lines for the addition of such items when they are wanted. Of the normal urinary constituents, urea and uric acid are those most frequently requiring quantitative estimation; while total solids, phosphates, chlorides, and sulphates are seldom asked for. A line is therefore left for the first two, while the remainder may be inserted when occasion demands. Under colour may be reported other physical characters, such as turbidity and froth. The spare lines can accommodate acetone, hæmoglobin, and other abnormal and unusual ingredients; a fresh heading for microscopical appearances, followed by six or eight blank lines, and again a new heading for remarks. This last, in reporting to a medical man, requires some tact, but the following may serve as an example of what may be said: 'Pus and albumin proportionate, and the large quantity of bladder epithelium points to cystitis.' Following are two kinds of forms in use, the second being the better.

(1)

REPORT ON SAMPLE OF URINE.

Received from
Address
Particulars of collection
Particulars of quantity

			Normal	Sample
Physical characters—				
Translucency			Transparent	
Consistency			Limpid	
Colour			Light golden	
Odour			Aromatic	
Froth			Temporary	
Reaction			Slightly acid	
Sp. Gr.			1·015 to 1·025	
Total Solids			800 to 1,000 gr. per diem	
Chemical	Normal Constituents	Chlorides	5 to 10 parts per 1,000, as NaCl	
		Sulphates	1·5 to 3 parts per 1,000, as SO_3	
		Phosphates	2 to 3 parts per 1,000, as P_2O_5	
		Urea	1·5 to 3 per cent.	
		Uric Acid	3 to 7 parts per 1,000	
Chemical	Abnormal Constituents	Albumin		
		Albumoses		
		Sugar		
		Bile		
		Bile pigments		
		Bile acids		
		Hæmoglobin		
Microscopical	Oxalates			
	Uric acid			
	Phosphates			
	Cystine			
	Leucin and Tyrosin			
	Cells (pus, blood, &c.)			
	Casts			
	Micro-organisms, as micrococci, bacteria, fungi, sarcinæ, spermatozoa			

(2)

Address

Date

EXAMINATION OF URINE.

Name of Patient
,, Doctor
Total Quantity in 24 hours
Colour, &c.
Deposit
Reaction
Specific Gravity
Urea (normal 1·5 to 2·5 per cent.)
Uric Acid (normal 0·3 to 0·7 per 1,000)
Bile Pigment
Indican
Albumin
Albumose
Sugar

MICROSCOPICAL APPEARANCES.

(*Six or eight lines left for this.*)

REMARKS.

(*Three or four lines left for this.*)

Test Reagents

Acid, acetic, 33 per cent. and glacial
Acid, carbolic
Acid, citric
Acid, hydrochloric
Acid, nitric
Acid, picric
Acid, salicylic
Acid, sulphuric
Ammonium chloride
Ammonium sulphate
Ammonium thiocyanate
Amylic alcohol
Barium chloride sol.
Barium hydroxide sol.
Bromine
Chloroform
Ether and ozonic ether
Fuchsin
Iron alum
Liq. ammon. fort.
Liq. potassæ
Liq. sod. chlorinatæ
Mercurous nitrate
Methyl violet
Methylene blue
Peptone
Phenylhydrazine hydrochloride
Phenolphthalein
Plumbi acetas
Potassii hydras
Potassium cyanide
Potassium ferrocy. sol.
Potassium iodide
Potassium sulphate
Soda tartarata
Sodii acetas
Sodii hydras
Sodium nitroprusside
Sol. arg. nit. (10 gr. ad ʒj.)
Sol. cupri sulph. (10 gr. ad ʒj.)
Sol. ferric chloride (neutral)
Spt. vini rect. or mineral-free s.v. meth.
Tr. guaiaci
Tr. iodi
Yeast (compressed)
Magnesia mixture — Dissolve magnes. carb. in a slight excess of hydrochloric acid, add one-third its bulk of liq. ammon. fort., then stir in ammonium chloride until the precipitate is dissolved.

Nearly all the salts and solutions are to be found in the B.P. and its Appendices, the remainder are easily obtained from any wholesale drug firm.

Apparatus

Albuminometer (Esbach's).
Balance
Beakers
Berlin porcelain dishes.

Burettes and stand.
Conical sediment-glasses ʒvj.
Filters (paper).
Flask (fitted with bent glass tube in the cork to allow escape of steam ; also a hole to receive the nozzle of a burette for use with Pavy's or Fehling'ssolution).
Flask, round-bottom, for nitrogen.
Funnels (glass).
Glycosometer (Gerrard's).
Litmus-paper (red and blue).
Measures, 50-c.c. and 1,000-c.c., each divided into 100 parts.
Metric weights and measures.
Microscope, slides, &c.
Nitrometer.
Pipettes (which deliver 5, 10, 15, 20, 30, and 50 c.c.).
Polarimeter.
Retort-stand.
Rods (glass).
Separating-funnel, 100-c.c.
Spectroscope.
Spirit-lamp, or Bunsen burner.
Test-tubes.
Test-tube stands.
Test-tube holders.
Ureometer (Southall's or Gerrard's).
Urine test-glasses.
Urinometer (Fletcher's).
Watch-glasses.

Physicians and others who have not the necessary accommodation may get all they want in the way of apparatus and reagents in the form of a portable case. Two of these are here described as typical examples. The first (made by Messrs. Burroughs Wellcome & Co., London) is a nickel-plated metal case, $5\frac{3}{4}$ inches by $2\frac{3}{4}$ inches by $1\frac{1}{4}$ inch, which may be carried in the pocket. It contains an Esbach's albuminometer, a

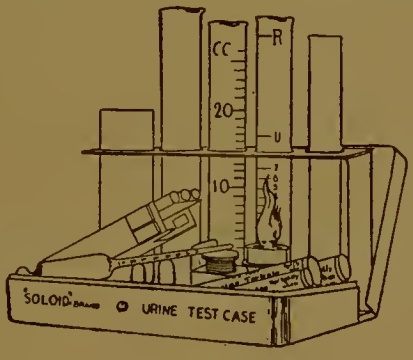

FIG. 21

urinometer, graduated measure, test-tubes and stand, a nickel-plated spirit-lamp, and 'Soloid' brand Fehling test, picric acid, potassium ferrocyanide, and citric acid in compressed form. The reagents are accurately weighed, so that they may be used (in appropriate instances) for quantitative as well as qualitative work. By means of the Esbach albuminometer the percentage of albumin may be ascertained ; and the quantity of urine required to discharge the blue colour of a definite measure of the Fehling test-solution will indicate the amount of grape-sugar present.

The next illustration shows a case and contents designed by Mr. W. Harrison Martindale, Ph.D. It is made of mahogany, and measures 6 inches by $2\frac{1}{2}$ inches by 4 inches. It contains the apparatus and reagents requisite

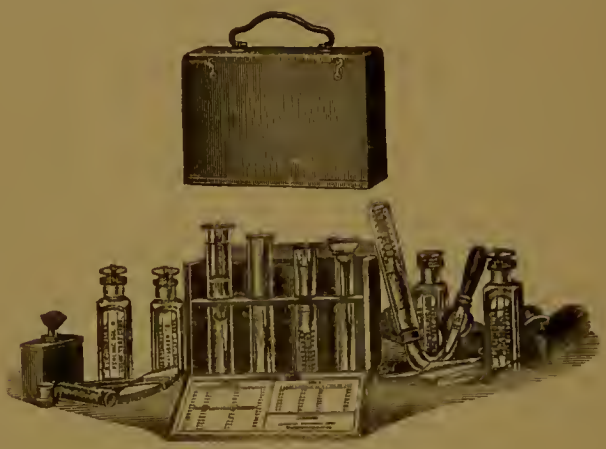

FIG. 22.

for the qualitative and quantitative examination of urine for albumin, glucose, and urea. The case contains an Esbach's albuminometer, graduated tube for sugar-determination, ureometer for urea-determination, urinometer for taking specific gravity, metal spirit-lamp with brass screw-top, three test-tubes, test-tube stand, test-tube brush, four stoppered bottles of reagents fitted with patent safety-stopper bands, funnel, graduated pipette, litmus-papers, filter-papers, cloth, calculation-tables (for albumin, sugar, and urea), book of directions, and charts for recording the results of the analysis.

Standard Test-solutions

Fehling's Solution

Solution of potassio-cupric tartrate, B.P.

No. 1

Sulphate of copper 34·64 grammes
Sulphuric acid 0·5 c.c.
Distilled water to make 500 c.c.

No. 2

Caustic soda 77 grammes
Tartarated soda 176 grammes
Distilled water to make 500 c.c.

Mix equal volumes, and 10 c.c. is equal to 0·05 gramme of diabetic sugar.

Pavy's Solution

Dissolve 20·4 grammes of Rochelle salt and the same weight of caustic potash in distilled water. Dissolve separately 4·158 grammes of cupric sulphate in more water, with heat; add this to the solution first prepared; when cold, add 300 c.c. of strong ammonia, and then make up with distilled water to a litre. Ten c.c. of this ammoniated cupric solution should have its colour exactly discharged by a portion of urine containing 0·005 gramme of glucose.

Standard Solutions for Phosphates

1. *Uranium solution.*—Dissolve 70 grammes of uranic nitrate in 900 c.c. of water, then ascertain its strength by making an analysis of 50 c.c. of the standard phosphate solution, and dilute with water so that 50 c.c. will correspond exactly to 50 c.c. of that solution: each c.c. will then be equal to 0·01 gramme P_2O_5.
2. *Standard phosphate solution.*—Dissolve 50·42 grammes of pure disodium hydrogen phosphate in 900 c.c. of water, and make up to a litre with water, when each c.c. will equal 0·01 gramme of P_2O_5.
3. *Sodium-acetate solution.*—A solution of 100 grammes of sodium acetate and 100 grammes of acetic acid made up to a litre with water.
4. A weak yellowish solution of ferrocyanide of potassium.

Standard Solutions for Chlorides

1. The volumetric solution of silver nitrate of the B.P.
2. Volumetric solution of ammonium thiocyanate. Dissolve 10 grammes of the thiocyanate in 1,000 c.c. of water, and adjust its strength until it agrees with the decinormal silver nitrate c.c. for c.c.
3. Strong solution of iron alum for indicator.

Standard Solution for Acidity

The decinormal volumetric solution of sodium hydroxide of the B.P.

Standard Solution for Uric Acid

Dissolve 1·578 gramme of potassium permanganate in 1,000 c.c. of water. Each c.c. = 0·00375 gramme of uric acid.

Solution of Sodium Hypobromite

1. Dissolve 100 grammes of purified sodium hydroxide in 250 c.c. of water.

2. Bromine.

When required mix 5 c.c. of (1) with 0·5 c.c. of (2); this will liberate the nitrogen from 1 c.c. of urine. Or use four times the quantities if 5 c.c. of urine is taken.

Special Reagents and Processes

The following are some processes and reagents which have been recommended by special workers, and which are grouped here for convenience of reference.

Almen's Albumin Reagent

Tannic acid . . .	4 grms.
Glacial acetic acid . .	2 c.c.
Distilled water . .	6 c.c.
Alcohol (50-per-cent.) to	200 c.c.

Dissolve.

Of this solution 10 c.c. is mixed with the same volume of urine, and the precipitated albumin filtered off. The nitrogen in 5 c.c. of the filtrate is determined by the Kjeldahl method, and a similar determination made with 5 c.c. of the original urine. The difference multiplied by 6·3 gives the amount of albumin and globulin precipitated.

Amann's Indican Test

To 20 c.c. of urine add a few drops of sulphuric acid and 5 c.c. of chloroform, then 5 c.c. of sodium-persulphate solution. Shake gently. The chloroform is coloured blue if indican is present, and the aqueous layer is red.

Baeyers' Acetone Test

Dissolve a few crystals of nitro-benzaldehyde in the urine by heating, cool, and add dilute soda solution in excess. If acetone is present this solution becomes yellow, green, and blue within ten minutes.

Bartley's Bile Test

Solution of ferric chloride containing free hydrochloric acid. A few drops of this added to the clear urine strikes a beautiful emerald-green colour if bile be present.

Chautard's Acetone Test

Decolorise solution of magenta with sulphurous acid. With 0·01 p.c. solutions of acetone the mixture becomes of a violet colour, and weaker solutions develop the colour on standing.

Crismer's Glucose Test

(As modified by A. H. Allen)

Mix equal volumes of the urine, liquor potassæ, and 1-in-1,000 solution of safranine. Boil, and if the urine contains more than 0·1 per cent. of glucose it will be decolorised, if less the red colour persists.

'One of the simplest and most certain clinical tests for glucose.'—Allen's 'Chemistry of Urine,' p. 84.

Fowler's Urea Test

The urine is mixed with seven times its volume of Squibb's chlorinated-soda solution, and after a few hours the specific gravity of

the mixture is taken. This is subtracted from the mean of the specific gravity before mixing, and the difference multiplied by 0·77 gives the percentage of urea.

Esbach's Albumin Reagent

(Gawalowski's Modification)

Picric acid . .	10 grms.
Citric acid . .	20 grms.
Dissolve in	
Water . . .	500 c.c.
And add	
Alcohol (95 per cent.)	350 c.c.
And sufficient	
Water to make .	1,000 c.c.

Gower's Glucose Test

Dilute the urine with an equal volume of liquor potassæ. Boil the upper half well, but not too long —a lemon tint corresponds with about 5 gr. of glucose per fl. oz.; a pale sherry, 10 gr.; a dark sherry, 15 gr.; and a port-wine tint, 20 gr. and upwards.

Haines's Glucose Reagent

Copper sulphate . . .	℥ss.
Distilled water . . .	℥ss.
Dissolve and add	
Glycerin	℥ss.
Then	
Solution of potash . .	℥v.
Mix.	

Used like Fehling's solution.

Hay's Bile Test

If sublimed sulphur is sprinkled upon urine which contains bile salts, the sulphur gradually sinks. Professor Matthew Hay states that this is a delicate test for bile salts, and that as little as 1 of the salts in 10,000 gives the reaction, which depends upon the power of bile salts to reduce surface-tension. The simplest method of carrying out the test (according to Drs. Beddard and Pembery) is to place the urine in a test-tube (1-inch diameter), and to throw some sublimed sulphur upon it. If any begins at once to fall in the urine, there is at least 1 part of bile salts per 10,000. When none falls at once, then, after waiting one minute, the test-tube is given a very gentle shake; if sulphur now begins to fall, there is at least 1 part of bile salts per 40,000; and so on for further dilutions. The urine must be free from air-bubbles, it must be clear, and if it is necessary to clear it, this must be done by filtration, and not by heat. The urine must be cooled to about 17° C. The test is ten times more sensitive than Pettenkofer's.

Heidenlang's Reagent

This is solid metaphosphoric acid, which, on addition to albuminous urine, coagulates the albumin.

Heintz's Uric-acid Process

Mix 100 c.c. of urine with 10 c.c. hydrochloric acid. Set aside for twenty-four hours, collect the crystals on a tared filter, wash with water, dry in a desiccator, and weigh.

Hopkins's Uric-acid Process

Add ammonium chloride 30 grammes to 100 c.c. of urine, dissolve, and neutralise with ammonia. Set aside for ten minutes to allow the acid ammonium urate to separate, collect it on a filter, wash with ammonium-sulphate solution (sat.), then rinse off with 100 c.c. of water, and add 20 c.c. sulphuric acid to the mixture. Warm to 60° C., and titrate with $\frac{N}{20}$ potassium permanganate. Each c.c. of permanganate equals uric acid 0·00375 gramme.

Tunnicliffe and Rosenheim improve the method by taking the well-washed uric-acid precipitate from the above process, and titrat-

ing it with $\frac{N}{20}$ piperidine solution (4·25 grammes piperidine per litre), with phenolphthalein as an indicator, the end-point being the formation of a purple colour. One c.c. of $\frac{N}{20}$ piperidine = 0·0084 gramme of uric acid.

Jaffe's Indole Test

Mix 10 c.c. each urine and hydrochloric acid, and add fresh liq. calc. chlorinat., drop by drop, until the blue colour is at its deepest. Shake with chloroform, which extracts the indigo.

Albumin, if present, should first be removed.

Johnson's Standard Colour Solution

Liq. ferri perchlor. fort. (B.P.) . . .	ʒj.
Acid. acetic. glacial. (B.P.) . . .	ʒiv.
Liq. ammoniæ (B.P.) .	ʒvj.
Aq. destillat. ad . .	℥iv.

Mix in the above order.

This solution is of the same depth of colour as that produced when a 1 gr. per oz. solution of glucose is boiled with picric acid and liquor potassæ.

Knop's Test

This is the hypobromite method of estimating urea.

Lieben's Reagent for Acetone

Potassium iodide . .	℈j.
Potash solution . .	ʒj.

Dissolve, boil, and float the urine on it. If acetone is present the precipitate of phosphates is yellow, and crystals of iodoform are seen.

McMunn's Indole Test

Equal parts of urine and hydrochloric acid (with a few drops of nitric acid) are mixed, boiled, and cooled. Then shake with chloroform, which draw off (the solution is violet) and examine in the spectroscope. An absorption-band before D and one after it are seen.

Albumin, if present, should first be removed.

Meissner's Hippuric-acid Method

Baryta-water is added in slight excess to 1 litre of urine, and excess of barium precipitated with sulphuric acid. Filter, neutralise with hydrochloric acid, and evaporate to a thick syrup, which mix with 150 c.c. alcohol. Set aside to precipitate, and filter. The filtrate contains hippuric acid. Evaporate to nearly crystallising-point and extract the hippuric acid with ether.

Millon's Reagent

Mercury . . .	10 grms.
Nitric acid, s.g. 1·185 .	25 grms.
Distilled water . .	25 c.c.

Dissolve by gentle heat, shaking frequently, and add to the following solution prepared in the cold :

Mercury . . .	10 grms.
Nitric Acid, s.g. 1·25 .	22 grms.

This gives a yellow coloration with albumin or urea, changing to red on heating.

Moss's Sugar Process

In Pavy's modification of Fehling's method, cupric oxide is reduced in presence of a large excess of ammonia, which prevents the precipitation of cuprous oxide. The temperature of the boiling liquid varies from about 70° C. to 90° C., and the rate of reduction varies to a corresponding extent. Mr. R. J. Moss overcomes this objection by using a much smaller quantity of ammonia, and conducting the titra-

tion under pressure, at the temperature of boiling water. The reduction of the cupric oxide is apparently instantaneous, and the results are very sharp and constant.

Nitro-propiol Test

Sodium orthonitro-phenyl propiolate is supplied under the trademark name 'Propiol' as a confirmatory test for sugar in urine, indigo-blue being formed by the reducing action of the sugar. If ¼ gr. of the salt is added to a mixture of 10 minims of urine and 3 dr. of water and boiled, the mixture assumes a deep-blue colour if sugar is present. If the colour does not appear quickly, continue the boiling for about five minutes before deciding on the result of the test.

Nylander's Glucose Reagent

Basic nitrate of bismuth	2·5 grms.
Rochelle salt . .	4 grms.
Soda solution, 8 p.c.	100 c.c.

Dissolve.

Mix 10 c.c. with 1 c.c. of the urine and boil. If glucose is present the liquid becomes black. It is better to filter through glass-wool before boiling.

This is an improvement of Böttger's test.

Oliver's Peptone Solution

Powdered peptone (Savory & Moore's) . .	ʒss.
Salicylic acid . . .	gr. iv.
Acetic acid . . .	ʒss.
Distilled water to . .	ʒviij.

Dissolve and filter repeatedly until transparent.

A test for bile acids, 60 minims of the solution being added to 20 minims of clear urine.

Oliver's Albumin Reagent

A mixture of equal parts of sodium-tungstate solution (1 to 4) and citric-acid solution (10 to 6).

This by contact precipitates albumin, peptone, mucin, and sometimes urates, but not alkaloids.

Pollacci's Albumin Reagent

Prepare a solution of tartaric acid, 1 gramme, corrosive sublimate 5 grammes, and sodium chloride 10 grammes, in 100 c.c. of distilled water, and add 5 c.c. of formaldehyde (40 per cent.) To test the specimen, put about 2 c.c. of the solution in a test-tube, then carefully add 3 to 4 c.c. of the urine, taking care that the two fluids do not mix. Should a ring immediately appear at the division of the two fluids, the urine contains pathological albumin. If the ring appears slowly after about ten to fifteen minutes, the urine is physiologically right, and the small quantities of albumin shown are normal.

The test is said to be sensitive to 1 in 370,000.

Purdy's Albumin Method

The specific gravity of the urine is raised 10 to 15 degrees by adding brine before boiling with 1 or 2 drops of acetic acid in the ordinary way.

Purdy-Fehling Solution

Copper sulphate .	4·742 grms.
Caustic potash .	23·50 grms.
Ammonia solution (s.g. 0·900) .	450 c.c.
Glycerin . .	38 c.c.
Distilled water to	1,000 c.c.

Dissolve the copper salt and glycerin in 200 c.c. of water, and the potash separately in as much; mix when cold, add the ammonia, and make up.

Thirty-four c.c. = 0·02 gramme grape-sugar.

Reynolds's Acetone Test

Add freshly precipitated yellow mercuric oxide to the urine, shake, and filter carefully; add ammonium-sulphate solution to the filtrate, and if acetone is present a black ring of mercury sulphide appears at the junction of the liquids.

Riegler's Uric-acid Reaction

To 5 c.c. of the urine add a few crystals of phosphomolybdic acid and 10 to 20 drops of soda solution. The presence of uric acid is indicated by the formation of a blue colour.

Roberts's Brine

Mix 1 pint of saturated solution of common salt with 1 oz. of hydrochloric acid, and filter. Pour the solution into the test-tube first, and the suspected albuminous urine above it.

Roberts's Nitric-acid Reagent

Dissolve 10 parts of Epsom salt in 13 parts of hot water, and filter. To 5 volumes of the solution add 1 volume of nitric acid s.g. 1·42.

The great density of this acid solution ensures that when the urine is poured upon it in the test-tube mixing is avoided, and the ring of albumin is clearly seen.

Spiegler's Reagent for Albumin

Mercuric chloride .	8 grms.
Tartaric acid . .	4 grms.
Glycerin . . .	20 c.c.
Water . . .	200 c.c.

Dissolve.

The urine is acidulated with acetic acid and filtered. Pour the reagent carefully on the surface of the filtrate. If albumin is present, a white ring is formed. Globulin and hemalbumose give a similar reaction, but not peptones. Sugar sometimes replaces glycerin in the reagent.

Rubner's Test for Lactose

Treat the urine with excess of lead acetate, filter, and add ammonia to the filtrate to form a permanent precipitate. Heat, and if a rose colour develops slowly, disappearing on standing, glucose is present; if not, and sugar has been found by copper reduction, it is lactose.

Tanret's Reagent for Albumin

Mercuric chloride .	1·35 grm.
Potassium iodide .	3·22 grms.
Distilled water to .	100 c.c.

Dissolve.

This gives a precipitate in albuminous urine acidulated with acetic acid. The reagent is also used volumetrically, but alkaloids and peptones are also precipitated by it.

Urobilin Test

(Roman and Delluc's)

Acidify 100 c.c. of the specimen with 10 drops of acetic acid. Shake well with 20 c.c. chloroform. To 2 c.c. of the chloroform solution add carefully 4 c.c. of 1-in-1,000 solution of zinc acetate in S.V.R., so that the liquids do not mix. Between the layers a green fluorescence is observable if urobilin be present, and on shaking the mixture acquires a rose tint.

Weyle's Test

When a solution of sodium nitro-prusside is added to dilute solution of creatinine followed by sodium-hydroxide solution, a red colour is produced, which changes to yellow on standing.

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